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? b 155, 5, 357
    04jun03 08:52:51 User208669 Session D2308.1
      $0.32   0.090 DialUnits File1
   $0.32 Estimated cost File1
   $0.03 TELNET
   $0.35 Estimated cost this search
   $0.35 Estimated total session cost   0.090 DialUnits

SYSTEM:OS - DIALOG OneSearch
 File 155:MEDLINE(R) 1966-2003/May W4
      (c) format only 2003 The Dialog Corp.
*File 155: Medline has been reloaded and accession numbers have
changed. Please see HELP NEWS 155.
 File   5:Biosis Previews(R) 1969-2003/Jun W1
      (c) 2003 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.
 File 357:Derwent Biotech Res. _1982-2003/Jun W2
      (c) 2003 Thomson Derwent & ISI
*File 357: File is now current. See HELP NEWS 357.
Alert feature enhanced for multiple files, etc. See HELP ALERT.

    Set  Items  Description
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? ds
Set    Items   Description
S1    307637   (G AND C) OR GC OR CG
S2     8290    TOBACCO(W)MOSAIC
S3      115    S1 AND S2
S4    288053   VECTOR OR VECTORS
S5       11    S3 AND S4
S6       10    RD (unique items)
S7    762971   RNA
S8     31810   S4 AND S7
S9      2410   S1 AND S8
S10   667597   CONTENT OR USAGE
S11      160   S9 AND S10
S12      143   RD (unique items)
S13     6075   S4(5N)S7
S14       12   S12 AND S13
S15  2864213   G + C
S16        0   "G + C"
S17    31037   G(2W)C
S18       44   S17 AND S13
S19       42   RD (unique items)
S20     3938   SPURIOUS
S21        0   S14 AND S20
S22        1   S13 AND S20
S23     2751   SINGLE(2W)RNA(W)(VIRUS OR VIRUSES)
S24     4206   SINGLE(W)STRAND?(W)RNA AND (VIRUS OR VIRUSES)
S25     4929   SSRNA
S26     6633   S23 OR S24 OR S25
S27      667   S4 AND S26
S28        4   S17 AND S27
? t s6/7/1-10
 6/7/1    (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
09041815  20336613  PMID: 10877820
 Rapid production of the major birch pollen allergen Bet v 1 in Nicotiana
benthamiana plants and its immunological in vitro and in vivo
characterization.
 Krebitz M; Wiedermann U; Essl D; Steinkellner H; Wagner B; Turpen T H;
Ebner C; Scheiner O; Breiteneder H
 Department of Pathophysiology, University of Vienna, Austria.
 FASEB journal - official publication of the Federation of American
Societies for Experimental Biology (UNITED STATES) Jul 2000, 14 (10)
 p1279-88, ISSN 0892-6638  Journal Code: 8804484
 Document type: Journal Article
 Languages: ENGLISH
 Main Citation Owner: NLM
 Record type: Completed
 Type I allergies are immunological disorders that afflict a quarter of
the world's population. Improved diagnosis of allergic diseases and the
formulation of new therapeutic approaches are based on the use of
recombinant allergens. We describe here for the first time the application
of a rapid plant-based expression system for a plant-derived allergen and
its immunological characterization. We expressed our model allergen Bet v
1, the major birch pollen allergen, in the tobacco-related species
Nicotiana benthamiana using a tobacco mosaic virus vector. Two weeks
postinoculation, plants infected with recombinant viral RNA containing the
Bet v 1 coding sequence accumulated the allergen to levels of 200 microg/g
leaf material. Total nonpurified protein extracts from plants were used for
immunological characterizations. IgE immunoblots and ELISA (enzyme-linked
immunoassay) inhibition assays showed comparable IgE binding properties for
tobacco recombinant (r) Bet v 1 and natural (n) Bet v 1, suggesting that
the B cell epitopes were preserved when the allergen was expressed in N.
benthamiana plants. Using a murine model of type I allergy, mice immunized
with crude leaf extracts containing Bet v 1 with purified rBet v 1 produced
in E. coli or with birch pollen extract generated comparable
allergen-specific IgE and IgG1 antibody responses and positive type I skin
test reactions. These results demonstrate that nonpurified Bet v 1
overexpressed in N. benthamina has the same immunogenicity as purified Bet
v 1 produced in E. coli or nBet v 1. We therefore conclude that this plant
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expression system offers a viable alternative to fermentation-based production of allergens in bacteria or yeasts. In addition, there may be a broad utility of this system for the development of new and low-cost vaccination strategies against allergy.
Record Date Created: 20000905
Record Date Completed: 20000905

6/7/2 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

03326886 BIOSIS NO.: 000072054990
FURTHER PROPERTIES OF PEANUT CLUMP VIRUS AND STUDIES ON ITS NATURAL
TRANSMISSION
AUTHOR: THOUVENEL J C; FAUQUET C
AUTHOR ADDRESS: LAB. VIROL., OFF. RECH. SCI. TECH. OUTRE-MER, B.P.V 51,
ABIDJAN, REPUBLIQUE DE COTE D'IVOIRE, AFR. OUEST.
JOURNAL: ANN APPL BIOL 97 (1). 1981. 99-108. 1981
FULL JOURNAL NAME: Annals of Applied Biology
CODEN: AABIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH
ABSTRACT: Purified preparations of particles of peanut clump virus (PCV) had A260/A280 values (corrected for light scattering) of 1.00. They contained rod-shaped particles with sedimentation coefficients of 183 S and 224 S, and a density in CsCl of 1.32 g/ml. PCV infected 36 spp. in 8 plant families. No serologicalrelationship was detected between PCV and barley stripe mosaic, beet necrotic yellow vein, Nicotiana velutina mosaic and tobacco mosaic viruses. PCV was seed-borne for 2 generations in groundnut (Arachis hypogaea) but was not seed-borne in great millet (Sorghum arundinaceum), Phaseolus mungo or Nicotiana benthamiana. Seedlings of groundnut, great millet and wheat (Triticum aestivum) became infected when grown in soil from groundnut fields with outbreaks of clump disease, and the infectivity of soil survived air-drying at 25.degree. C for 3 mo. Groundnut seedlings became infected when grown in sterilized soil contaminated with washed roots of naturally-infected S. aurundinaceum but not in soil to which roots of naturally infected groundnut or shoots of infected groundnut were added, or in which mechanically inoculated groundnut seedlings were grown at the same time. The patchy distribution of PCV in a crop was related to the infectivity of the soil for groundnut and to the presence of Polymyxa graminis resting spores which could be detected in the roots of S. arundinaceum bait seedlings, but not in those of groundnut. PCV is apparently transmitted by a vector that is resistant to air-drying and closely associated with S. arundinaceum roots. For these reasons P. graminis is thought to be the vector of PCV.

6/7/3 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0307781 DBR Accession No.: 2003-09566 PATENT
New uncapped RNA molecule of a positive strand replicating RNA virus, useful as RNA transformation vectors for producing phenotypically transformed plants that are e.g. pest or pathogen resistant, or herbicide tolerant - vector expression in host cell useful for constructing transgenic plant
AUTHOR: LINDBO J A; POGUE G P; TURPEN T H
PATENT ASSIGNEE: LINDBO J A; POGUE G P; TURPEN T H 2002
PATENT NUMBER: US 20020164803 PATENT DATE: 20021107 WPI ACCESSION NO.:
2003-220044 (200321)
PRIORITY APPLIC. NO.: US 949317 APPLIC. DATE: 20010907
NATIONAL APPLIC. NO.: US 949317 APPLIC. DATE: 20010907
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An uncapped RNA molecule of a single-component single-stranded (+) sense RNA virus, which is capable of infecting a host plant cell, is new. The uncapped RNA molecule comprises a viral replication element, an exogenous RNA segment, and no base, a single base or a sequence of bases located at the 5' terminus of the viral sequence. DETAILED DESCRIPTION - An uncapped RNA molecule of a single-component single-stranded (+) sense RNA virus, which is capable of infecting a host plant cell, comprises: (a) a cis-acting viral replication element obtained from a single-component (+) strand RNA plant virus; (b) no base, or a single base, or a sequence of bases located at the 5' terminus of the viral sequence; and (c) an exogenous RNA segment capable of expressing its function in a host plant cell, where the exogenous RNA segment is located in a region of the uncapped RNA molecule able to tolerate the exogenous RNA segment without disrupting RNA replication of the uncapped RNA molecule, and where the uncapped RNA molecule is capable of replication in the absence of a trans-acting viral replication element. INDEPENDENT CLAIMS are also included for the following: (1) modifying a host plant cell phenotypically by introducing into the cell the uncapped RNA molecule, where the exogenous RNA segment confers a detectable trait in the host cell, thus modifying the host cell; and (2) a DNA transcription vector comprising cDNA having one strand complementary to the uncapped RNA molecule capable of infecting a host plant cell. BIOTECHNOLOGY - Preferred RNA Molecule: The exogenous RNA segment of the uncapped RNA molecule codes for a peptide or protein. This exogenous RNA segment comprises an antisense RNA, a structural RNA, a regulatory RNA, or RNA having catalytic properties. The RNA virus is a tobamo virus, specifically a tobaco mosaic virus. In particular, the uncapped RNA molecule is encapsidated with viral coat protein. The host plant is a

dicotyledonous plant cell, particularly Nicotiana. The uncapped RNA molecule capable of infecting a host plant cell has no cap at the 5' terminus of the viral sequence. This uncapped RNA molecule may also comprise: (a) the entire genome of a single-component, single-stranded (+) sense RNA virus, without a cap sequence; and (b) an exogenous RNA segment, capable of expressing its function in a host plant cell, where the exogenous RNA segment is inserted into the genome of the RNA virus under the control of a subgenomic promoter.The uncapped RNA molecule further comprises one or more nucleotide base molecules inserted at the 5' terminus of the viral sequence. USE - The uncapped RNA molecule is useful as RNA transformation vectors for modifying a plant host cell. In particular, the uncapped RNA molecule is useful for producing phenotypically transformed plants under field or greenhouse growth conditions to produce plants that are e.g. pest resistant, pathogen resistant, herbicide tolerant, or with modified growth habit and modified metabolic characteristics (e.g. production of commercially useful peptides or pharmaceuticals in plants). (37 pages)

6/7/4 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
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0305383 DBR Accession No.: 2003-07168 PATENT
New modified staphylococcal enterotoxin derived from a native disulfide loop-containing pyrogenic toxin, useful for non-specifically enhancing an immune function and as a vaccine against toxic shock syndrome or food poisoning - recombinant toxin protein production via plasmid expression in host cell for use in recombinant vaccine and gene therapy
AUTHOR: MARSHALL M J; SHIEL P J; BERGER P H; BOHACH G A; BOHACH C H
PATENT ASSIGNEE: IDAHO RES FOUND INC 2002
PATENT NUMBER: WO 200283169 PATENT DATE: 20021024 WPI ACCESSION NO.:
2003-058608 (200305)
PRIORITY APPLIC. NO.: US 283720 APPLIC. DATE: 20010413
NATIONAL APPLIC. NO.: WO 2002US11619 APPLIC. DATE: 20020411
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A modified pyrogenic toxin derived from a native disulfide loop-containing pyrogenic toxin, is new, where the modified toxin comprises a disulfide loop having no more than 10 amino acids. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an expression vector comprising a nucleic acid sequence encoding the modified pyrogenic toxin; and (2) a host cell transformed with the expression vector. BIOTECHNOLOGY - Preferred Modified Toxin: The modified toxin is derived from a native disulfide loop-containing pyrogenic toxin which is a staphylococcal toxin or a streptococcal toxin. The staphylococcal toxin is a type A, B, C, D, E, G or H staphylococcal enterotoxin. The native disulfide loop-containing pyrogenic toxin is specifically a staphylococcal enterotoxin type C1 (SEC1). The staphylococcal enterotoxin C may be a staphylococcal enterotoxin C1 or C2, staphylococcal enterotoxin C-MNCopeland, staphylococcal enterotoxin C-4446, staphylococcal enterotoxin C-bovine, staphylococcal enterotoxin C-canine, or staphylococcal enterotoxin C-ovine. The disulfide loop region contains no more than 3 or 8 amino acid residues. The modification comprises a deletion of between 4 - 18 amino acid residues within the disulfide loop region. The modified toxin has a fever-inducing activity or an emetic response-inducing activity decreased by about 100-fold in comparison to a native toxin. The modified toxin comprises an N-terminal domain of a staphylococcal toxin and a C-terminal domain of a second staphylococcal toxin. It further comprises an exogenous sequence of between 1 - 30 amino acid residues located within the disulfide loop region. The exogenous sequence comprises a sequence of alanine amino acid residues. Preferred Vector: The expression vector comprises a tobacco mosaic virus vector. Preferred Host Cell: The host cell is a plant cell from Nicotiana benthamiana or Chenopodium quinoa. Preparation: The modified toxin is prepared by standard molecular biology and recombinant techniques. ACTIVITY - Immunostimulant; Antibacterial. No biological data is given. MECHANISM OF ACTION - Vaccine; Gene therapy. USE - The modified pyrogenic toxin, that is a staphylococcal enterotoxin, is useful for non-specifically enhancing an immune function and for vaccination against diseases such as toxic shock syndrome and food poisoning. ADMINISTRATION - The dosage may range from about 1 - 1000 micrograms/kg of body weight. Administration can be via injection or infusion by intravenous, intramuscular, intracerebral, intraperitoneal, intracerebrospinal, subcutaneous, parenteral, intraocular, intraarticular, intrasynovial, intrathecal, oral, topical or inhalational routes. It can also be given by intratumoral, peritumoral, intralesional, or perilesional routes. ADVANTAGE - The modified toxin has a fever-inducing activity or an emetic response-inducing activity decreased by about 100-fold in comparison to a native toxin. EXAMPLE - The structural gene for SEC1 from Staphylococcus aureus strain MNDON was used as native SEC1. A 1.0 Kb HindIII-BamHI (3'-5') fragment containing sec + mndon was sub-cloned from pMIN146 into the multiple cloning site of the 5.6 Kb pALTER (RTM) -1 phagemid vector. This vector was then used to transform Escherichia coli TG1. Mutagenesis was performed on sec + mndon obtained from E. coli TG1. Six mutant SEC1 toxins containing sequential deletions within the loop region were generated. (67 pages)

6/7/5 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
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0305228 DBR Accession No.: 2003-07013 PATENT

New DNA construct comprising a modified nucleic acid molecule having at least 80% homology to a desired trait DNA, useful for imparting resistance to plants against a variety of pathogens, e.g. viruses, bacteria, fungi or viroids - transgenic plant construction via plasmid expression in host cell for disease-resistance

AUTHOR: GONSALVES D; FERMIN-MUNOZ G A
PATENT ASSIGNEE: CORNELL RES FOUND INC 2002

PATENT NUMBER: WO 200286146 PATENT DATE: 20021031 WPI ACCESSION NO.:
2003-093146 (200308)
PRIORITY APPLIC. NO.: US 286075 APPLIC. DATE: 20010424
NATIONAL APPLIC. NO.: WO 2002US13377 APPLIC. DATE: 20020424
LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A DNA construct comprising a modified nucleic acid molecule having a nucleotide sequence which is at least 80%, but less than 100%, homologous to two or more desired trait DNA molecules and which imparts the desired trait to plants transformed with the DNA construct, is new. DETAILED DESCRIPTION - A DNA construct comprises a modified nucleic acid molecule having a nucleotide sequence which is at least 80%, but less than 100%, homologous to two or more desired trait DNA molecules and which imparts the desired trait to plants transformed with the DNA construct, is new. Each of the desired trait DNA molecules relative to the modified nucleic acid molecule have a nucleotide sequence similarity value which differs by no more than 3 percentage points. INDEPENDENT CLAIMS are also included for the following: (1) a DNA expression vector comprising the DNA construct above; (2) a host cell transformed with the DNA construct above; (3) a transgenic plant transformed with the DNA construct above; (4) a transgenic plant seed transformed with the DNA construct above;(5) imparting a trait to plants comprising transforming a plant with a DNA construct above, or by planting a transgenic plant seed and propagating a plant from the planted transgenic plant seed; (6) preparing a modified nucleic acid molecule suitable to impart multiple traits to a plant; and (7) determining whether multiple desired traits can be imparted to plants by a single modified nucleic acid molecule. BIOTECHNOLOGY - Preferred DNA Construct: The modified nucleic acid has a sequence selected from a 217, 201, 219, 202, 207, 134, 291, 260, and 4 sequences of 216 bp given in the specification. The modified nucleotide sequence has a length that is insufficient to impart the desired trait to plants transformed with the DNA construct. The DNA construct further comprises a silencer DNA molecule operatively coupled to the modified nucleic acid molecule, where the modified nucleic acid molecule and the silencer DNA molecule collectively impart the desired trait to plant transformed with the DNA construct. The silencer DNA molecule is selected from a viral cDNA molecule, a jellyfish green fluorescence protein encoding DNA molecule, a plant DNA molecule, and a viral gene silencer, and encodes an RNA molecule which is (non-)translatable. The DNA construct further comprises several modified nucleic acid molecules, each directed to a different trait than the other modified nucleic acid molecules in the DNA construct, where at least some of the modified nucleic acid molecules have a nucleotide sequence which is at least 80%, but less than 100%, homologous to several desired trait DNA molecules, and at least some of the desired trait DNA molecules relative to its respective modified nucleic acid molecule have a nucleotide sequence similarity value differing by no more than 3 percentage points. Some of the trait DNA molecules are derived from a plant viral genome and the trait is viral disease resistance, where the trait DNA molecules derived from a plant viral genome consists of a DNA molecule encoding a coat protein, a DNA molecule encoding replicase, a DNA molecule not encoding a protein, a DNA molecule encoding a viral gene product, or their combinations. The trait DNA molecules may also be derived from a plant virus selected from tomato spotted wilt virus, impatiens necrotic spot virus, groundnut ringspot virus, potato virus Y, potato virus X, tobacco mosaic virus, turnip mosaic virus, tobacco etch virus, papaya ringspot virus, tomato mottle virus, tomato yellow leaf curl virus, arabis mosaic virus, grapevine rupestris stem pitting associated virus-1, rupestris stem pitting associated virus-1, grapevine leafroll-associated virus 3, 4, 8, 1, 5, 7 or 2, grapevine virus A, grapevine trichovirus B, grapevine virus B, and their combinations. Alternatively, at least some of the train DNA molecules are plant DNA molecules which effect color and/or enzyme production, and the trait is a plant genetic trait. The DNA construct further comprises a promoter sequence, and a termination sequence, which are operatively coupled to the modified nucleic acid molecule. The DNA construct effects post-transcriptional gene silencing within plants. Each of the desired trait DNA molecules relative to the modified nucleic acid molecule have a nucleotide sequence similarity value and each of these similarity values differs by no more than 2 or 1 percentage point(s). Preferred Host Cell: The host cell is selected from a bacterial cell, a virus, a yeast cell, and a plant cell, preferably the host cell is a plant cell. Preferred Transgenic Plant: The transgenic plant is selected alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, papaya, sugarcane, Arabidopsis thaliana, Saintpaulia, petunia, pelargonium, poinsettia, chrysanthemum, carnation, or zinnia. Preferred Method: The method of imparting traits to a plant further comprises propagating the progeny of the transgenic plant. Preparing a modified nucleic acid molecule for imparting multiple traits to a plant, comprises: (a)

identifying a plurality of desired traits to be imparted to plants, where the desired traits are imparted by desired trait DNA molecules having nucleotide sequences; (b) selecting as a reference nucleotide sequence, one nucleotide sequence from among the desired trait DNA molecules identified; and (c) modifying the reference nucleotide sequence to form a modified nucleic acid molecule which is at least 80%, but less than 100%, homologous to the nucleotide sequences of the desired trait DNA molecules identified, where molecule have a nucleotide sequence similarity value which differs by no more than 3 percentage points, preferably by no more than 2 or 1 percentage point(s). The method further comprises identifying a region in the desired trait DNA molecules having a high degree of sequence similarity, where modification is carried out using that region of one of the desired trait DNA molecules as the reference nucleotide sequence. The region of the reference nucleotide sequence selected has a nucleotide sequence that is most dissimilar or similar from all desired trait DNA molecules. The method also includes operatively coupling several modified nucleic acid molecules together to form a fusion gene imparting the desired trait to plants transformed with the fusion gene and having a sufficient length to impart the desired trait to plants transformed with the fusion gene. Determining whether multiple desired traits can be imparted to plants by a single modified nucleic acid molecule, comprises: (a) identifying several desired traits to be imparted to plants, where the desired traits are imparted by desired trait DNA molecules having nucleotide sequences; (b) selecting as a reference nucleotide sequence, one nucleotide sequence from among the desired trait DNA molecules identified; (c) modifying the reference nucleotide sequence to form a modified nucleic acid molecule; and (d) determining whether the modified nucleic acid molecule is at least 80%, but less than 100%, homologous to the desired trait DNA molecules identified and whether each of the desired trait DNA molecules relative to the modified nucleic acid molecule have a nucleotide sequence similarity value which differs by no more than 3 percentage points. ACTIVITY - Antibacterial; Fungicide; Virucide; Insecticide. A three quarters tomato spotted wild virus (TSWV) gene was modified to make it more homologous to the groundnut ringspot virus (GRSV) N gene nucleotide sequence. The newly created sequence, when compared with its parental three quartersN TSWV-BL gene sequence, has the following changes: one insertion and one deletion, plus 22 base changes. About 27% of the transgenic lines analyzed showed a good level of resistance against either TSWV or GRSV. MECHANISM OF ACTION - Gene therapy. USE - The DNA construct is useful for imparting resistance to plants against a wide variety of pathogens including viruses, bacteria, fungi, viroids, phytoplasmas, nematodes and insects. The DNA construct may also be used to impart a desired genetic trait to the plant, such as desired color, enzyme production (or cessation of enzyme production), and plant hormones. EXAMPLE - 1 microl of each oligonucleotides corresponding to every single construct were mixed in combinations, and were polymerase chain reaction (PCR) amplified under the following conditions: 55 cycles of denaturation at 94degreesC for 30 seconds, annealing at 52degreesC for 30 seconds, and extension at 72degreesC for 30 seconds. 2.5 microl of this assembly was mixed with primers for PCR amplification under the following conditions: 35 cycles of denaturation at 92degreesC for 30 seconds, annealing at 65degreesC for 30 seconds and extension at 72degreesC for 2 minutes. All gene fragments were digested with an excess of BamHI and XhoI for not less than 12 hours. Digested fragments were excised from agarose gels and column purified for ligation into the BamHI/XhoI cloning site of vector pEPJ86GFP, where a transcription fusion with GFP gene was created under the control of a double 35S promoter. Five independent recombinant plasmids per construct were sequenced, plasmids with identical sequence to the computer-generated gene fragments were chosen for digestion and subsequent subcloning into pGA482G. digestion with KpnI and HindIII renders subcloning fragment with the GFP gene fused to the synthetic three quarters N gene fragment, and under the control of the 35S promoter. After checking by PCR and restriction analysis, recombinant derivatives of pGA482G were sequenced again before transformation into Agrobacterium tumefaciens LBA4404. (191 pages)

6/7/6 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0302121 DBR Accession No.: 2003-03906 PATENT
Purifying proteins or non-proteinaceous small molecules of interest by infecting a host with a virus/viral expression vector, useful for the commercial production of proteins or small molecules in both prokaryotes and eukaryotes - recombinant protein production via plasmid expression in host cell
AUTHOR: GLEBA Y; BASCOMB N; NEGROUK V
PATENT ASSIGNEE: ICON GENETICS INC 2002
PATENT NUMBER: WO 200268927 PATENT DATE: 20020906 WPI ACCESSION NO.:
2002-707029 (200276)
PRIORITY APPLIC. NO.: US 262466 APPLIC. DATE: 20010118
NATIONAL APPLIC. NO.: WO 2002US1676 APPLIC. DATE: 20020118
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Purifying proteins or non-proteinaceous small molecules of interest, comprising infecting a host with a virus or the viral expression vector (particles of the virus form complexes with the protein or small molecule via the peptide recognition sequence), is new. DETAILED DESCRIPTION - Purifying proteins or non-proteinaceous small molecules of interest, comprising infecting a host with a virus or the viral expression vector (particles of the virus form complexes with the protein or small molecule via the

peptide recognition sequence). The method comprises: (a) providing a virus having on its surface at least one recognition peptide, or preparing a viral expression vector which upon infection of an organism yields particles of the virus; (b) obtaining a host that produces the protein or small molecule of interest independent from due to presence of the virus within it; and (c) infecting the host with the virus or the viral expression vector where particles of the virus form complexes with the protein or small molecule via the peptide recognition sequence, followed by harvesting the complexes of the virus and protein from the host, or containing a lysate or solution of the host with the virus (particles of the virus form complexes with the protein or small molecule via the peptide recognition sequence), and the protein or small molecule is separated from the solution. INDEPENDENT CLAIMS are also included for: (1) a method of production and purifying a protein of interest, comprising: (a) providing a plant virus containing a coat protein (a surface of the coat protein contains a recognition peptide), or providing a viral expression vector or pro-vector which upon processing and infection of a plant yields particles of the virus; (b) genetically modifying a plant host to produce the protein of interest; and (c) infecting the host with the virus or the viral expression vector where particles of the virus form complexes with the protein or small molecule via the peptide recognition sequence, followed by harvesting the complexes of the virus and protein from the host, or containing a lysate or solution of the host with the virus (particles of the virus form complexes with the protein or small molecule via the peptide recognition sequence), and the protein or small molecule is separated from the solution; (2) a method of visualizing or localizing a protein or non-proteinaceous small molecule of interest, comprising: (a) providing a virus having an affinity for the protein or small molecule and an affinity for a visualization agent; (b) obtaining a host that produces the protein or small molecule of interest; (c) contacting the host or a preparation with the virus; (d) exposing the contacted host or preparation with the visualization agent; and (e) detecting presence or locale of the protein or small molecule; (3) a non-human host comprising virus particles having on their surfaces at least one recognition peptide, the particles having a protein or non-proteinaceous small molecule bound directly or indirectly via the recognition peptide; and (4) a composition of matter comprising virus particles having on their surfaces at least one recognition peptide, the particles having a protein or non-proteinaceous small molecule bound directly or indirectly via the recognition peptide. BIOTECHNOLOGY - Preferred Method: The method of purifying a protein or non-proteinaceous small molecule of interest further comprises separating the protein or small molecule of interest from the virus particles. The recognition peptide is a part of a native coat protein, and the protein of interest is fused to an affinity peptide that binds the recognition peptide (the affinity peptide comprises a single-chain fragment (scFv) of an antibody or an antibody that binds the recognition peptide). The recognition peptide is a FLAG polypeptide or a polyhistidine polypeptide, and the protein of interest is fused to an affinity peptide that binds the recognition peptide (the affinity peptide comprises a single-chain fragment (scFv) of an antibody or an antibody that binds the FLAG polypeptide or the polyhistidine polypeptide). Alternatively, the recognition peptide is an affinity peptide, an scFv of an antibody, or an antibody that binds a small non-proteinaceous molecule. Additionally, the recognition peptide is a polypeptide sequence introduced into a coat protein of the virus and has an affinity to the protein of interest. Step (a) further comprises preparing a viral expression vector that contains a transgene encoding the protein of interest or another protein, the production of which results in production of the protein or small molecule of interest in the host. Step (b) further comprises transforming the host with a transgene encoding the protein or another protein the production of which results in production of the protein or small molecule of interest (the transgene encodes a fusion protein comprising the protein of interest linked via a cleavable linkage to an affinity peptide that binds the recognition peptide). The cleavable linkage comprises an enterokinase site, a cyanogens bromide-sensitive site or a cleavable intein or a fragment. The virus is a bacteriophage, a tobacco mosaic virus or an adenovirus. Step (a) further comprises a virus infecting plants, yeasts or animals, or preparing a viral expression vector which is a plant, yeast or animal viral vector. Alternatively, step (a) comprises providing a bacteriophage that infects a bacterium or preparing a viral expression vector that is a bacterial viral vector. The host is a plant cell, a plant tissue or a plant. The host is alternatively a bacterium, yeast or an animal cell. The surface of the virus contains more than one recognition peptide having the same or different affinities. Step (d) in the method of (1) further comprises separating the protein of interest from the virus. USE - The methods and compositions of the present invention are useful for the large-scale commercial production of proteins and small molecules in any prokaryotic and eukaryotic system. EXAMPLE - A cDNA copy of TMV (tobacco mosaic virus) inserted into pBR322 plasmid known as TMV304 was used. 937bp KpnI-NcoI fragment with 3' end of TMV was cut from TMV304 and inserted into pICxxxx vector. This produced the plasmid pICyyyy. The 21-nucleotide sequence encoding the heptapeptide Thr-Leu-Ile-Ala-His-Pro-Gln that has affinity to streptavidin was inserted into the gene for the TMV CP by PCR (polymerase chain reaction) site-specific mutagenesis. The modified gene was inserted back into the plasmid resulting in the recombinant DNA pICzzzz. Infectious RNA copy of the modified virus was transcribed in vitro and inoculated into young plants. Two weeks after inoculation virus was isolated both from TMV and TMVf23 inoculated plants. Recovery of TMV and TMVf23 was very good with both yielding 2 mg of virus per 1 g of

leaf tissue.(31 pages)

6/7/7 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0292059 DBR Accession No.: 2002-13906
Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine - virus infection vaccine production, vector expression in transgenic plant, chimeric peptide, nucleoprotein and alfalfa-mosaic virus coat protein
AUTHOR: YUSIBOV V; HOOPER DC; SPITSIN S; FLEYSH N; KEAN RB; MIKHEEVA T
; DEKA D; KARASEV A; COX S; RANDALL J; KOPROWSKI H
CORPORATE AFFILIATE: Thomas Jefferson Univ Thomas Jefferson Univ
CORPORATE SOURCE: Koprowski H, Thomas Jefferson Univ, Biotechnol Fdn Labs, 1020 Locust St,Room 346 JAH, Philadelphia, PA 19107 USA
JOURNAL: VACCINE (20, 25-26, 3155-3164) 2002
ISSN: 0264-410X
LANGUAGE: English
ABSTRACT: AUTHOR ABSTRACT - A new approach to the production and delivery of vaccine antigens is the use of engineered amino virus-based vectors. A chimeric peptide containing antigenic determinants from rabies virus glycoprotein (G protein) (amino acids 253-275) and nucleoprotein (N protein) (amino acids 404-418) was PCR-amplified and cloned as a translational fusion product with the alfalfa mosaicvirus (AIMV) coat protein (CP). This recombinant CP was expressed in two plant virus-based expression systems. The first one utilized transgenic Nicotiana tabacum cv. Samsun NN plants providing replicative functions in transfor full-length infectious RNA3 of AIMV (NF1-g24). The second one utilizedNicotiana benthamiana and spinach (Spinacia oleracea) plants using autonomously replicating tobacco mosaic virus (TMV) lacking native CP (Av/A4-g24).Recombinant virus containing the chimeric rabies virus epitope was isolatedfrom infected transgenic N. tabacum cv. Samsun NN plants and used for parenteral immunization of mice. Mice immunized with recombinant virus were protected against challenge infection. Based on the previously demonstrated efficacy of this plant virus-based experimental rabies vaccine when orally administered to mice in virus-infected unprocessed raw spinach leaves, we assessed its efficacy in human volunteers. Three of five volunteers who had previously been immunized against rabies virus with a conventional vaccine specifically responded against the peptide antigen after ingesting spinach leaves infected with the recombinant virus. When rabies virus non-immune individuals were fed the same material, 5/9 demonstrated significant antibody responses to either rabies virus or AIMV. Following a single dose of conventional rabies virus vaccine, three of these individuals showed detectable levels of rabies virus-neutralizing antibodies, whereas none of five controlsrevealed these antibodies. These findings provide clear indication of thepotential of the plant virus-based expression systems as supplementary oralbooster for rabies vaccinations. DERWENT ABSTRACT: All plants used in these experiments were grown and maintained in a controlled BL2P greenhouse. For animal experiments, recombinant viral construct NF1-g24 was produced in transgenic tobacco (Nicotiana tabacum cv. Samsun NN) plants expressing the alfalfa-mosaic virus (AIMV) P1 and P2 (P12) replicase genes. Three upper leaves of each plant were inoculated with a mixture of in vitro transcription products of recombinant constructs in 2 volumes (v/v) of FES buffer (sodium-pryophosphate 1% (w/v), macaloid 1% (w/v), celite 1% (w/v), glycine 0.5 M, K2HPO4 0.3 M, pH 8.5, with phosphoric acid). Inoculum was applied by gentle rubbing on leaves after abrading the leaf surface with carborundum. Recombinant virus was isolated 12-14 days after the inoculum was applied. Briefly, leaf tissue was homogenized and the sap separated from cell debris by centrifugation. Virus particles were selectively precipitated using 5% PEG. To study the immunogenicity of the experimental rabies vaccine administered orally, three lots of spinach plants were inoculated with in vitro transcription products of Av/A4-g24, harvested 12-14 days later, washed, analyzed for the presence of rabies virus antigen, packaged in 150 g doses and fed to human volunteers(10 pages)

6/7/8 (Item 6 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0196716 DBR Accession No.: 96-08096 PATENT
New nucleic acid encoding fusion protein including tobamo virus coat protein - recombinant antigen preparation by tobacco-mosaic virus coat protein fusion protein gene expression in transgenic plant for use in e.g. malaria recombinant vaccine
AUTHOR: Turpen T H; Reinl S J; Grill L K
CORPORATE SOURCE: Vacaville, CA, USA.
PATENT ASSIGNEE: Biosource-Technol. 1996
PATENT NUMBER: WO 9612028 PATENT DATE: 960425 WPI ACCESSION NO.: 96-222012 (9622)
PRIORITY APPLIC. NO.: US 324003 APPLIC. DATE: 941014
NATIONAL APPLIC. NO.: WO 95US12915 APPLIC. DATE: 951006
LANGUAGE: English
ABSTRACT: Nucleic acid encoding a fusion protein, consisting of a tobamo virus coat protein fused to a protein of interest at a fusion point, is claimed. The fusion may be an amino or carboxy terminus fusion, or an internal fusion. Preferably, the fusion joint contains a leaky start or stop codon, which results in the production of both the fusion protein and the virus coat protein from a single virus vector, the protein of interest is an antigen, and the coat protein is a tobacco-mosaic virus coat protein, especially the 17.5 kD protein. Also claimed are: (a) a

recombinant plant virus genome containing the nucleic acid; (b) the fusion protein; (c) a recombinant plant virus in which the coat protein is encoded by the nucleic acid; and (d) plant cells containing the nucleic acid. The antigen fusion protein may be used to raise antibodies for use in e.g. immunoassays or in vaccines to protect against parasitic infection e.g. malaria. The fusion protein can be produced economically and at a high level in plants. (53pp)

6/7/9 (Item 7 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
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0163730 DBR Accession No.: 94-06281 PATENT
Expression of foreign gene in transgenic plant - e.g. tobacco-mosaic virus replication-associated enzyme expression in tobacco cell or transgenic plant for crop improvement or protein production
PATENT ASSIGNEE: PCC-Technol. 1994
PATENT NUMBER: JP 6038772 PATENT DATE: 940215 WPI ACCESSION NO.: 94-094835 (9412)
PRIORITY APPLIC. NO.: JP 91339485 APPLIC. DATE: 911129
NATIONAL APPLIC. NO.: JP 91339485 APPLIC. DATE: 911129
LANGUAGE: Japanese
ABSTRACT: A new method for foreign gene (I) expression in plant cells or plants involves: (a) introducing a gene encoding a protein (II) participating in plant single-stranded RNA virus replication in plant cell chromosomes into plant cells or plants to form transformed cells or plants; (b) inserting (I) into a vector containing a recognition site for (II) and being replicated by (II), and lacking the sequence encoding the GDD sequence common to RNA-dependent RNA-polymerase (EC-2.7.7.6) or highly homogeneous 11 amino acids around the sequence; and (c) introducing the vector into the plant cells or plant produced in (a). The new method is simple and facilitates production of useful proteins by the plant cells or transgenic plants. In an embodiment, (I) encodes a sweetener or a pharmaceutical or confers temp.-resistance, herbicide resistance or increased yield on the plants. In an example, tobacco-mosaic virus enzyme RNA was used to transform tobacco protoplasts, which were then transformed with vector plasmid pLDR22, plasmid pLDR24, plasmid pLDR27, plasmid pLDR28 or plasmid pLDR29. pLDR28 expressed recombinant protein. (18pp)

6/7/10 (Item 8 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
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0157075 DBR Accession No.: 93-15127 PATENT
Plant virus vector for foreign gene expression in transgenic plant - contains foreign gene downstream of virus coat protein gene, linked by a read-through sequence; application to pharmaceutical preparation
PATENT ASSIGNEE: Kanebo 1993
PATENT NUMBER: WO 9320217 PATENT DATE: 931014 WPI ACCESSION NO.: 93-336923 (9342)
PRIORITY APPLIC. NO.: JP 92351970 APPLIC. DATE: 921208
NATIONAL APPLIC. NO.: WO 93JP408 APPLIC. DATE: 930331
LANGUAGE: Japanese
ABSTRACT: A plant virus vector contains a foreign gene attached downstream of the virus outer coat protein gene via a sequence which induces readthrough. Also claimed are plasmids containing this vector, and preferably a phage T7 or PM promoter. The virus vector may be a DNA virus or an RNA virus, such as cauliflower-mosaic virus, gemini virus, tobacco-mosaic virus, cucumber-mosaic virus, etc. More specifically, the sequence inducing read-through is UAGCAAUUA, TAGCAATTA, UAACAAUUA,
TAACAATTA, UGACAAUUA, TGACAATTA, UAGCARYYA or TAGCARYYA, where R = A or
G, Y = C, U or T, or their DNA equivalents. The vector is used to infect a suitable host plant, such as tobacco (Nicotiana tabacum), tomato (Lycopersicon esculentum), cauliflower (Brassica oleracea botrytis), cabbage (Brassica oleracea capitata), cucumber (Cucumis sativus), etc., which then expresses the foreign protein as part of the viral particles. The protein can be isolated from the virus produced. This method can be used for the production of pharmaceuticals such as human erythrocyte growth factor, angiotensin-converting-enzyme (EC-3.4.15.1), enkephalin, calcitonin or corticotrophin. (39pp)
? t s14/7/7 8 10
14/7/7 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.
0304044 DBR Accession No.: 2003-05829 PATENT
Producing protein in recombinant expression system involves predicting secondary structure of RNA encoding a protein and increasing free energy for the secondary structure by modifying sequence of DNA encoding the RNA - vector-mediated gene transfer, expression in host cell and bioinformatic software for nucleic acid vaccine and gene therapy
AUTHOR: WEINER D B; YANG J
PATENT ASSIGNEE: WEINER D B; YANG J 2002
PATENT NUMBER: US 20020123099 PATENT DATE: 20020905 WPI ACCESSION NO.:
2003-066795 (200306)
PRIORITY APPLIC. NO.: US 971806 APPLIC. DATE: 20011004
NATIONAL APPLIC. NO.: US 971806 APPLIC. DATE: 20011004
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Producing (M) a protein by translation of mRNA from heterologous DNA sequence (HDS), involves predicting the secondary structure of mRNA transcribed from a native HDS, modifying native HDS, where mRNA transcribed from modified HDS has

a secondary structure with increased free energy compared to mRNA transcribed from native HDS and using modified HDS for protein production, is new. DETAILED DESCRIPTION - Producing (M) a protein in a recombinant expression system by translation of mRNA from heterologous DNA sequence (HDS), involves predicting the secondary structure of mRNA transcribed from a native HDS, modifying native HDS to produce modified HDS, where mRNA transcribed from modified HDS has a secondary structure with increased free energy compared to mRNA transcribed from native HDS and using modified HDS for protein production. INDEPENDENT CLAIMS are also included for the following: (1) an injectable pharmaceutical composition (PC) comprising a nucleic acid (NA) molecule (I) that includes a modified coding sequence encoding a protein operably linked to regulatory elements, where the modified coding sequence comprises a higher AT or AU content relative to the AT or AU content of the native coding sequence; and (2) a recombinant viral vector (II) comprising (I). BIOTECHNOLOGY - Preferred Method: The secondary structure of the mRNA transcribed from a native heterologous DNA sequence is predicted using a computer and a computer program, and is modified by increasing the AT content of the coding sequence at the 5' end of the coding sequence, within 200 or 150 or 100 nucleotides from the initiation codon such that mRNA transcribed from it has an increased AU content. Preferred Composition: In PC, the modified coding sequence encodes an immunogen such as a pathogen derived proteins, a cancer antigen, autoimmune disease associated protein, or their immunogenic fragments, or a fusion protein that includes the above proteins or their immunogenic fragments, or encodes a non-immunogenic therapeutic protein such as cytokines, growth factors, blood products or enzymes. The modified coding sequence comprises dispersed modifications which are up to 100, 150 or 200 bases in length alternating with regions of native coding sequence. The sequence comprises a higher AT or AU content in the first 100, 150 or 200 bases relative to the AT or AU content of the native nucleic acid sequence. ACTIVITY - Immunosuppressive; Antirheumatic; Cytostatic; Neuroprotective; Antiarthritic; Antidiabetic; Antithyroid; Antiulcer; Antipsoriatic; Virucide; Antiparasitic; Antiallergic. No biological data given. MECHANISM OF ACTION - Gene therapy. USE - (M) is useful for producing a protein in a recombinant expression system, preferably a cell free in vitro transcription and translation system, an in vitro cell expression system, a DNA construct used in direct DNA injection, or a recombinant vector for delivery of DNA to an individual (claimed). PC is useful for eliciting broad immune responses against a target protein, i.e. proteins specifically associated with pathogens such as viruses, parasites, allergens, or the individual's own abnormal cells. PC confers a broad based protective immune response against hyperproliferative cells that are characteristic in hyperproliferative diseases including all forms of cancer and psoriasis. PC is also useful for treating individuals suffering from autoimmune diseases including rheumatoid arthritis, multiple sclerosis, Sjogren's syndrome, insulin dependent diabetes mellitus, autoimmune thyroiditis, Crohn's disease, ulcerative colitis, and psoriasis. EXAMPLE - The addition of a leader sequence to minimize free energy in the West Nile virus Capsid mRNA resulted in enhanced protein expression and immune response. To enhance the transcription and translation efficiency of transgenes, the human IgE leader sequence was added to the 5' upstream of open reading frame (orf) sequences. The addition of a sequence encoding the human IgE leader sequence containing codons that were less prevalently utilized in humans (WNBy-DJY construct (yeast codon)) resulted in a predicted secondary structure for the mRNA with an increased free energy value, relative to the secondary structure for the mRNA without the leader sequence (WNVwt construct (wild type)), or relative to the secondary structure for the mRNA encoding a leader sequence optimized with human codons (WNVh-DJY construct (human codon)). The construct encoding the leader sequence containing codons that were less prevalently utilized in humans (yeast optimized) yielded a higher level of protein than did the construct encoding the leader sequence containing human optimized codons, as determined by immunoprecipitation of radiolabeled in vitro translated proteins. The codons more prevalently used by yeast were, in general, AU rich and the codons more prevalently used by Homo sapiens were, in general, more GC rich. DNA plasmid injection into mouse muscle induced an antigen-specific, CD4+, Th cell-dependent immune response, as determined by intracellular interferon-gamma (IFN-gamma/flow cytometry analysis. The CD4+ Th cell-dependent, intracellular IFN-gamma production was quantitated by flow cytometry. Splenocytes isolated from pWNVy-DJY (pWNVCy)-immunized mice, expressed higher levels IFN-gamma, upon stimulation with in vitro translated Cp protein, than did the splenocytes isolated from pWNVh-DJY (pWNVCh)-immunized mice. (25 pages)

14/7/8 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0299591 DBR Accession No.: 2003-01375 PATENT
New monopartite or bipartite RNA viral vector, useful for silencing or expressing foreign genes in a plant host, comprises a modified tobravirus RNA-1 and/or RNA-2 genes - transgenic construction via plasmid expression in host cell for altered alkaloid content and secondary metabolite
AUTHOR: ROBERTS P D; VAEWHONGS A A; KUMAGAI M H
PATENT ASSIGNEE: LARGE SCALE BIOLOGY CORP 2002
PATENT NUMBER: WO 200259335 PATENT DATE: 20020801 WPI ACCESSION NO.:
2002-599799 (200264)
PRIORITY APPLIC. NO.: US 771035 APPLIC. DATE: 20010125
NATIONAL APPLIC. NO.: WO 2002US2498 APPLIC. DATE: 20020125
LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A monopartite or bipartite ribonucleic acid (RNA) viral vector (I) comprising a modified tobravirus RNA-1 with an inserted foreign RNA sequence operably linked to the 3'-end of the stop codon of the RNA sequence that codes for a 16k Da cysteine-rich protein of RNA-1, where the bipartite vector additionally comprises a tobravirus RNA-2, is new. DETAILED DESCRIPTION - The modified tobravirus RNA-2 may comprise one or more promoter-gene construct inserted in place of the 2C gene without removing the 2b gene of the virus, where a subgenomic promoter is operably linked to the 5'-end of a second foreign RNA sequence. INDEPENDENT CLAIMS are also included for: (1) expressing (M1) one or more foreign genes in a plant host by infecting a plant host with the RNA viral vector, where the foreign gene is expressed in the plant host; (2) silencing (M2) one or more endogenous plant host genes using (M1), where the expression of the foreign RNA sequence causes silencing of an endogenous plant host gene; (3) simultaneously silencing (M3) a plant host gene and expressing a foreign gene by infecting a host with the bipartite RNA viral vector, where the first foreign RNA sequence causes silencing of an endogenous gene of a plant host, and the second foreign RNA is expressed in the plant host, or where the second foreign RNA sequence causes silencing of an endogenous gene of a plant host, and the first foreign RNA is expressed in the plant host; (4) altering (M4) an alkaloid content in a plant host by infecting a plant host with (I); (5) a plant host infected by a viral RNA vector; (6) a plant host with an altered alkaloid content prepared by (M4); (7) compiling (M5) a plant functional gene profile, comprising: (a) preparing a library of DNA or RNA sequences from a donor plant, and constructing recombinant viral nucleic acids comprising an unidentified nucleic acid insert obtained from the library in either a positive sense or an antisense orientation, where the recombinant viral nucleic acids are obtained from a tobravirus; (b) infecting a plant host with one or more recombinant viral nucleic acids; (c) transiently expressing the unidentified or recombinant nucleic acid in the plant host; (d) determining one or more phenotypic or biochemical changes in the plant host; (e) identifying an associated trait relating to a phenotypic or a biochemical change; (f) identifying the recombinant viral nucleic acid that results in one or more changes in the plant host, or determining the sequence of the unidentified nucleic acid insert; (g) repeating steps (b)-(f) until at least one nucleic acid sequence associated with the trait mentioned is identified, where a functional gene profile of the plant host or of the plant donor is compiled; (8) changing (M6) the phenotype or the biochemistry of a plant host by employing (M1), where the foreign RNA sequence is expressed transiently in the plant host; and (9) determining the presence of a trait in a plant host by employing steps (a)-(d) of (M5), and comparing one or more biochemical or phenotypic traits to an uninfected plant host. BIOTECHNOLOGY - Preferred Viral Vector: The modified tobravirus RNA-2 of the bipartite RNA viral vector further comprises Not I, Pst I, and Xho sites. The foreign RNA is either a complete open reading frame or a partial open reading frame and codes for a protein or a part of a protein, such as Nop 10-like small nucleolar ribonucleoprotein, DEAD box RNA helicase, putrescine N-methyl transferase, methionine synthase, PRP 19-like spliceosomal protein, CRS2 protein, or guanine tri phosphate (GTP)-binding protein. The RNA sequence is obtained from any member of a library of RNA sequences taken from a eukaryotic or prokaryotic species. The vector is a silencing vector or an expression vector. Preferred Method: The above methods further comprises allowing the viral vector to infect the plant systemically. The foreign gene mentioned in the method of altering the alkaloid content in a plant host, encodes for all or part of putrescine N-methyltransferase. USE - (I) is useful in the expression of foreign genes that may be important in genetic engineering of agricultural crops. (I) and (M1-M6) are also useful in silencing endogenous plant host genes and in simultaneously silencing and expressing foreign genes in a plant host, thus, expanding the number of biological products that could be produced in plants. These biological products are secondary metabolites that contribute to tastes, scents and colors in food. These also serve as pharmaceuticals (e.g. morphine, vinblastine, taxol) and as defense compounds for plants. EXAMPLE - A deoxyribonucleic acid (DNA) template for ribonucleic acid (RNA) transcription was made by digesting the plasmid pLSB-1 PDS (+) with SmaI to linearize it at the 3'-end of the virus. Infectious RNA transcripts were made using components of the mMessage mMachine large scale in vitro transcription kit in a total volume of 4.3 mul. RNA-1 template transcriptions were done using 0.4 mul 10 x Transcription buffer, 2.0 mul 2 x Ribonucleotide Mix, 0.2 approximately ml 30 mM GTP, 1.3 mul DNA template, and 0.4 mul T7 RNA polymerase enzyme mix. The mixture was inoculated 1-2 hours at 37 degreesC, then used to inoculate N. benthamiana. The RNA transcripts were mixed with 50 mul FES (7.5 g/L glycine, 10.5 g/L dibasic potassium phosphate, 10 g/L sodium pyrophosphate, 10 g/L bentonite, 10 g/L celite), then pipetted on the top surface of two opposite leaves of the plant. Transcript RNA was manually rubbed into the leaves. Inoculated plants were maintained in an outdoor greenhouse. When N. benthamiana were inoculated with the RNA-1 pLSB-1 PDS (+) alone, there was bleaching of systemic leaves, starting at 2-2.5 weeks post-inoculation. Thus, pLSB-1 PDS (+) acted as a monopartite silencing vector. Unlike previous viral vectors developed for gene silencing in plants, this construct did not contain an additional subgenomic promoter. The phytoene desaturase inhibitor RNA was expressed on a subgenomic RNA which was operationally linked to the endogenous cyclic AMP receptor protein (CRP) RNA.(103 pages)

14/7/10 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0291022 DBR Accession No.: 2002-12869 PATENT
New nucleic acid sequence for controlling expression of inserted sequence, useful e.g. in gene therapy, comprises specific sequence of A boxes and C motifs - new construct useful for transgenic animal and transgenic plant construction and gene therapy
AUTHOR: BLIND M; FAMULOK M
PATENT ASSIGNEE: NASCACELL GMBH 2002
PATENT NUMBER: WO 200224931 PATENT DATE: 20020328 WPI ACCESSION NO.:
2002-352008 (200238)
PRIORITY APPLIC. NO.: DE 1046913 APPLIC. DATE: 20000921
NATIONAL APPLIC. NO.: WO 2001EP10905 APPLIC. DATE: 20010920
LANGUAGE: German
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Nucleic acid sequence (I), for expressing a sequence (II) inserted into it, comprises, in the 5' to 3' direction: C1 motif; A1 box; A2 box; C2 motif; A3 box and terminator, where C1 and C2 together form a helix; A1 and A2 have 0-100 bases and A3 has 0-20 bases. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) expression system (ES1) comprising (I); (2) expression system (ES2) comprising promoter, C1, A1, inserted RNA, A2, C2, A3 and polyU (RNA polymerase-III terminator); (3) vector containing (I), ES1 or ES2; (4) cells containing (I), ES1 or 2, or the vector of (3); and (5) transgenic animal or plant containing the cells. BIOTECHNOLOGY - Preferred Materials: Preferably the A1 and 2 boxes each contain 5-15 bases and A3 0-9 bases, while C1 and C2 contain at least 10, best 20, bases, forming a double helix of at least 10 base pairs. The terminator is for RNA polymerase-III (Pol-III) and comprises at least four U (for RNA) or T (for DNA). (I) may also include a promoter, especially for Pol-III, e.g. the 5S RNA, U6 sn RNA or tRNA promoters and a nucleic acid inserted between A1 and A2 boxes. The nucleic acid may be functional (e.g. an aptamer; intramer; aptazyme, or allosteric center from an aptamer or ribozyme, particularly one that interacts with its target by a mechanism other than complementary base pairing). Preferred Hosts: Cells are eukaryotic, especially mammalian (including human) and transgenic organisms are mammals (preferred), fish, insects or nematodes, also a wide variety of (crop) plants. USE - (I), also expression systems and vectors that include them, are used for target validation and identification, in gene therapy, for inhibiting specific proteins in transgenic organisms, e.g. to increase nutrient content of plants, and in screening programs, e.g. to identify antagonists to nucleic acid ligands. ADVANTAGE - (I) contains all the features needed for expression of functional nucleic acid at a high level and in a compartment-specific manner, in any selected tissue. Expression systems that contain (I) are active in host cells without requiring additional proteins for induction of expression. EXAMPLE - The expression construct PH1 comprises a double helix formed from C1 and C2 motifs (including two mismatches, separated by 9 base pairs); an A1 box of 13 bases, an A2 box of 12 bases and an A3 box of one base, attached to a U5 terminator. An aptamer, D28 (see PNAS, 96 (1999) 3606) was cloned into PH1, between A1 and A2 boxes. The resulting construct could bind to Sepharose-immobilized CD18cyt peptide with an affinity comparable with that for D28 itself. (68 pages)

? t s19/7/1 5 13 29 33
19/7/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14552890 22315512 PMID: 12427277
%(G+C) variation and prediction by a model of bacterial gene transfer and codon adaptation.
Buckley Cedric O; Stephens Desmond; Herring Patricia A; Jackson Julius H
Theoretical & Computational Biology Group, Michigan State University, East Lansing, Michigan 48824, USA. jhjacksn@msu.edu
Omics - a journal of integrative biology (United States) 2002, 6 (3) p259-72, ISSN 1536-2310 Journal Code: 101131135
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The %(G + C) of bacterial genomes ranges from 25% in Mycoplasma to 75% in Micrococcus. Our model for horizontal gene flow enabled a theoretical study of the adaptation of relative codon frequency to match the pattern of the tRNA set of a new host. This study explored the dynamic relationship of %(G + C) to vectors of relative codon frequency (F(gamma)), relative amino acid coding frequency (F(alpha)), and absolute codon frequency (F(|gamma|)) in chromosomes of nine, fully sequenced bacterial genomes that varied widely in %(G + C). At constant F(alpha), the theoretical maximum average range possible was %(G + C) = 37.4 +/- 0.9%. In simulations of F(gamma) adaptation to a new host following hypothetical gene transfer, we modeled %(G + C) as a function of F(gamma) and F(alpha). The simulation revealed that %(G + C) is dependent on F(gamma) and F(alpha) in an explicit relationship described in this paper. We conclude that (1) F(gamma) and F(alpha) determine %(G + C), and (2) the degree of adaptation of %(G + C) in a transferred gene depends upon the degree of F(gamma) equilibration and the similarity of F(alpha) of the transferred gene to that of the new host.
Record Date Created: 20021112
Record Date Completed: 20030312

19/7/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10521574 96332659 PMID: 8730865
mRNA sequences influencing translation and the selection of AUG initiator

codons in the yeast Saccharomyces cerevisiae.
Yun D F; Laz T M; Clements J M; Sherman F
Department of Biochemistry, University of Rochester, School of Medicine and Dentistry, New York 14642, USA.
Molecular microbiology (ENGLAND) Mar 1996, 19 (6) p1225-39, ISSN 0950-382X Journal Code: 8712028
Contract/Grant No.: R01 GM12702; GM; NIGMS; T32 GM07098; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The secondary structure and sequences influencing the expression and selection of the AUG initiator codon in the yeast Saccharomyces cerevisiae were investigated with two fused genes, which were composed of either the CYC7 or CYC1 leader regions, respectively, linked to the lacZ coding region. In addition, the strains contained the upf1-delta disruption, which stabilized mRNAs that had premature termination codons, resulting in wild-type levels. The following major conclusions were reached by measuring beta-galactosidase activities in yeast strains having integrated single copies of the fused genes with various alterations in the 89 and 38 nucleotide-long untranslated CYC7 and CYC1 leader regions, respectively. The leader region adjacent to the AUG initiator codon was dispensable, but the nucleotide preceding the AUG initiator at position -3 modified the efficiency of translation by less than twofold, exhibiting an order of preference A > G > C > U. Upstream out-of-frame AUG triplets diminished initiation at the normal site, from essentially complete inhibition to approximately 50% inhibition, depending on the position of the upstream AUG triplet and on the context (-3 position nucleotides) of the two AUG triplets. In this regard, complete inhibition occurred when the upstream and downstream AUG triplets were closer together, and when the upstream and downstream AUG triplets had, respectively, optimal and suboptimal contexts. Thus, leaky scanning occurs in yeast, similar to its occurrence in higher eukaryotes. In contrast, termination codons between two AUG triplets causes reinitiation at the downstream AUG in higher eukaryotes, but not generally in yeast. Our results and the results of others with GCN4 mRNA and its derivatives indicate that reinitiation is not a general phenomenon in yeast, and that special sequences are required.
Record Date Created: 19960920
Record Date Completed: 19960920

19/7/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07717079 93172351 PMID: 8382299
Transcriptional activity and mutational analysis of recombinant vesicular stomatitis virus RNA polymerase.
Sleat D E; Banerjee A K
Department of Molecular Biology, Cleveland Clinic Foundation, Ohio 44195-5178.
Journal of virology (UNITED STATES) Mar 1993, 67 (3) p1334-9, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AI-26585; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The 241-kDa large (L) protein of vesicular stomatitis virus (VSV) is the multifunctional catalytic component of the viral RNA polymerase. A protocol has been developed for the synthesis of recombinant L protein that will support viral mRNA synthesis in vitro. COS cells were transfected with a transient expression vector (pSV-VSL1 [M. Schubert, G. G. Harmison, C. D. Richardson, and E. Meier, Proc. Natl. Acad. Sci. USA 82:7984-7988, 1985]) which contains the simian virus 40 late promoter for the transcription of a cDNA copy of the L protein of the Indiana serotype of VSV. Cytoplasmic extracts of these cells efficiently transcribed VSV mRNAs in vitro in conjunction with N protein-RNA template purified from virus and recombinant phosphoprotein synthesized in Escherichia coli. mRNA synthesis was completely dependent upon addition of both bacterial phosphoprotein and extracts from cells transfected with the L gene. Extracts from mock-transfected cells or from cells transfected with the expression vector alone did not support VSV RNA synthesis. RNA synthesis was proportional to the concentration of cell extract used, with an optimum of 0.2 mg/ml. Rhabdoviruses and paramyxoviruses contain a highly conserved GDNQ motif which was mutated in the transfected L gene. All constructs with mutations within the core GDN abrogated transcriptional activity except for the mutant containing GDD, which retained 25% activity. Conserved amino acid changes outside of the core GDN and changes corresponding to other paromyxovirus and rhabdovirus L proteins retained variable transcriptional activity. These findings provide experimental evidence that the GDN of negative-strand, nonsegmented RNA viruses is a variant of the GDD motif of plus-strand RNA viruses and of the XDD motif of DNA viruses and reverse transcriptases.
Record Date Created: 19930323
Record Date Completed: 19930323

19/7/29 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
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0305739 DBR Accession No.: 2003-07524 PATENT
Improving the genetic stability of foreign dna sequence to be inserted into single-stranded rna virus recombinant vectors comprises inducing mutation of the foreign DNA sequence to have uniform G/C ratio over the total foreign DNA sequence - recombinant yellow-fever virus, Venezuelan-horse-encephalitis virus, rubella virus or coxsackie virus

vector-mediated gene transfer and expression in host cell
AUTHOR: BAE Y S; KIM D Y; KIM G T; LEE S G
PATENT ASSIGNEE: CREAGENE INC 2002
PATENT NUMBER: KR 2002066048 PATENT DATE: 20020814 WPI ACCESSION NO.:
2003-145037 (200314)
PRIORITY APPLIC. NO.: KR 6229 APPLIC. DATE: 20010208
NATIONAL APPLIC. NO.: KR 6229 APPLIC. DATE: 20010208
LANGUAGE: KR
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A method for improving the genetic
stability of a foreign DNA sequence to be inserted into single-stranded RNA virus recombinant vectors is provided, where the genetic stability of the foreign DNA sequence within single-stranded RNA virus recombinant vectors can be significantly improved. DETAILED DESCRIPTION - The method for improving the genetic stability of a foreign DNA sequence to be inserted into single-stranded RNA virus recombinant vectors comprises inducing mutation of the foreign DNA sequence to have uniform G/C ratio over the total foreign DNA sequence, in which the single-stranded RNA virus recombinant vector is selected from the group consisting of Yellow fever virus vector, Venezuelan equine encephalitis virus vector, Rubella virus vector, and Coxsackievirus vector; the uniform G/C ratio can be accomplished by increasing the amount of G/C in the foreign DNA sequence; and the G/C ratio of the foreign DNA sequence to the total foreign DNA sequence is 40% or more.(1 pages)

19/7/33 (Item 7 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
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0284727 DBR Accession No.: 2002-06574 PATENT
New modified RNA virus of genus Morbillivirus, useful as vaccine for protecting humans against Morbillivirus infection, comprises mutations and/or deletions which reduce repression normally caused by V protein - useful for recombinant vaccine for immunization against RNA virus infection
AUTHOR: PARKS C L
PATENT ASSIGNEE: AMERICAN CYANAMID CO 2002
PATENT NUMBER: WO 200200694 PATENT DATE: 20020103 WPI ACCESSION NO.:
2002-139896 (200218)
PRIORITY APPLIC. NO.: US 213655 APPLIC. DATE: 20000623
NATIONAL APPLIC. NO.: WO 2001US19806 APPLIC. DATE: 20010621
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated, recombinantly-generated,
nonsegmented, negative-sense, single-stranded RNA virus (I) of the genus Morbillivirus having a mutation in the region corresponding to amino acids 112-134 of Morbillivirus V protein, especially amino acids 113 and 114, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also
included for the following: (1) an immunogenic composition (II) comprising (I); (2) reducing the repression caused by a V protein of the genus Morbillivirus, by inserting a mutation in the region corresponding to amino acids 112-134 (especially 113 and 114) of Morbillivirus V protein; (3) an isolated nucleotide sequence encoding a Morbillivirus V protein which has been modified by inserting a mutation in the region corresponding to amino acids 112-134 of a Morbillivirus V protein; (4) a composition comprising a transcription vector comprising an isolated nucleic acid molecule encoding a genome or antigenome of a Morbillivirus, where the portion of the nucleic acid molecule encoding the V protein has been modified to insert a mutation in the region corresponding to amino acids 112-134 of Morbillivirus V protein, together with an expression vector which comprises an isolated nucleic acid molecule encoding the trans-acting proteins N, P and L necessary for encapsidation, transcription and replication, where upon expression an infectious Morbillivirus is produced; and (5) producing an infectious Morbillivirus, by transforming, infecting or transfecting host cells with at least two vectors as above, and culturing the host cells under conditions which permit the co-expression of these vectors so as to produce the infectious Morbillivirus. BIOTECHNOLOGY - Preferred Virus: (I) is measles, canine distemper, rinderpest, pestedes-petits ruminants, dolphin Morbillivirus or phocine distemper virus. (I) has mutation at position 113 and/or 114. (I) further comprises a mutation in or deletion of a portion of a C-terminal region corresponding to amino acids 231-299 of the measles virus V protein, canine distemper virus V protein, rinderpest virus V protein, or corresponding to amino acids 231-303 of the rinderpest virus V protein. The mutation in the C-terminal region is at 233 and 234, from arginine to alanine or aspartic acid. The deletion is chosen from the deletion of amino acids 232-299, 279-299, 267-299, 250-299, 243-299 and 236-299. The deletion extends upstream from the C-terminal region and is from amino acid 229-299. (I) further comprises an attenuating mutation in the 3' genomic promoter region chosen from nucleotides 26 (A to T), 42 (A to T or A to C) and nucleotide 96 (G to A), where these nucleotides are presented in positive strand, antigenomic, message sense, and an attenuating mutation in the RNA polymerase gene chosen from nucleotide changes which produce changes in amino acid residues 331 (I to T), 1409 (A to T), 1624 (T to A), 1649 (R to M), 1717 (D to A), 1936 (H to Y), 2074 (Q to R) and 2114 (R to K). (I) also comprises an attenuating mutation chosen from: (a) for the N gene, nucleotide changes which produce changes in an amino acid residues 129 (Q to K), 148 (E to G) and 479 (S to T); (b) for the P gene, nucleotide changes in amino acid residues 225 (E to G), 275 (C to Y), 439 (L to P); (c) for the C gene, nucleotide changes which produce changes in amino acid residues 73 (A

to V), 104 (M to T) and 134 (S to Y); and (d) for the F gene-end signal, the change at nucleotides 7243 (T to C), where these nucleotides are presented in positive strand, antigenomic and message sense. Preferred Composition: (II) further comprises an adjuvant. ACTIVITY - Virucide. MECHANISM OF ACTION - Vaccine. No supporting data is given. USE - (II) is useful for immunizing an individual to induce protection against nonsegmented, negative-sense, single-stranded RNA virus of the genus Morbillivirus (claimed). ADMINISTRATION - Administered by parenteral, oral or topical, preferably intranasal route. Dosage not specified. EXAMPLE - The measles virus N, P and L protein expression clones were each prepared from infected-cell total RNA by reverse transcription and polymerase chain reaction (PCR) amplification (reverse transcription (RT)-PCR) with gene-specific primers, followed by cloning into an appropriate T7 RNA polymerase-dependent expression vector. Vero cells were infected with the Edmonston wild-type strain of measles virus, and when about 70% or more of the cells exhibited a cytopathic effect, RNA was prepared. RT-PCR was performed with avian myoblastosis virus RT and Pwo polymerase. Amplified DNA fragments were cloned into a T7 expression plasmid. Cloned DNAs were checked by cycle-sequencing and nucleotide substitution errors were corrected by oligonucleotide mutagenesis. The initial V protein expression clone was prepared by PCR amplification from an Edmonston wild-type full-length cDNA clone using primers flanking the V protein coding region. The amplified DNA was cloned into the T7 expression vector and the additional G nucleotide residue required to generate the V gene frame shift was added at the editing site by oligonucleotide-directed mutagenesis. Wild-type and mutant V protein expression vectors were also prepared with an influenza virus hemagglutinin (HA) epitope tag. The T7 vector plasmid was modified to include a sequence that included an initiation codon and encoded the HA epitope tag followed by a polylinker. The V protein coding region was cloned with the HA tag at the amino terminus. This served to replace the V protein initiator methionine codon, resulting in the generation of a plasmid, designated pMV-haV-wt. V protein mutants were prepared in the pMV-haV-wt backbone by oligonucleotide-directed or deletion mutagenesis. This modified V protein vector (pMV-haV-wt) also retained the base substitutions that prevented expression of C protein. Testing pMV-haV-wt in minireplicon experiments revealed that the presence of the N-terminal HA tag had no detectable effect on the ability of V protein to repress minireplicon activity (data not shown). Mutations introduced into pMV-haV-wt were directed at specific sequence motifs. One of the mutation resulted in a truncated V protein that lacked the unique V protein C-terminus containing the cysteine residues (amino acids 231-299 were deleted) (pMV-haV-1). The second mutant haV-5 contained substitution mutations at amino acids 113 and 114. Analysis of the mutant V protein vectors in a minireplication assay indicated that two vectors (pMV-haV-1 and pMV-haV-5) had diminished ability to repress minireplicon activity. (93 pages)

? t s22/7

22/7/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

07752294 93207781 PMID: 8457354

Improved design of riboprobes from pBluescript and related vectors for in situ hybridization.

Witkiewicz H; Bolander M E; Edwards D R

Department of Orthopedics, Mayo Clinic, Rochester, MN 55905.

BioTechniques (UNITED STATES) Mar 1993, 14 (3) p458-63, ISSN 0736-6205 Journal Code: 8306785

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The pBluescript family of plasmids and phagemids are sophisticated multi-purpose cloning vectors that allow convenient production of single-stranded sense and anti-sense RNA probes corresponding to DNA sequences inserted into a large multiple cloning site array. We have observed that in many applications sense (control) probes generated from genes cloned into pBluescript II KS(-) give high background signals on in situ hybridization to human tissue sections. Our studies indicate that this spurious hybridization is due to sequences contained within both strands of the multiple cloning site between the SmaI and SacI sites that are similar to human 28S rRNA. This information is useful in construct design in order to minimize nonspecific background problems, as demonstrated by in situ hybridization of sense and anti-sense probes corresponding to a portion of human stromelysin-3 to sections of human lung carcinoma.

Record Date Created: 19930423

Record Date Completed: 19930423

? t s28/7/1 3 4

28/7/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

07719672 93174945 PMID: 8438576

Reovirus protein lambda 3 is a poly(C)-dependent poly(G) polymerase.

Starnes M C; Joklik W K

Department of Microbiology, Duke University Medical Center, Durham, North Carolina 27710.

Virology (UNITED STATES) Mar 1993, 193 (1) p356-66, ISSN 0042-6822 Journal Code: 0110674

Contract/Grant No.: 1P01 CA30246; CA; NCI; 2 P3O CA14236; CA; NCI; R01 AI08909; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed
Reovirus protein lambda 3 has been isolated from cells infected with two recombinant vaccinia viruses into the TK gene of which the reovirus serotype3 strain Dearing L1 genome segment under the control of the bacteriophage T7 RNA polymerase promoter, or the T7 polymerase gene itself, had been cloned. Highly purified protein lambda 3 does not transcribe double-stranded reovirus RNA into single-stranded RNA, or plus-stranded reovirus RNA into minus-stranded RNA, but it does transcribe poly(C) into poly(G). It prefers Mn2+ to Mg2+. A polymer consisting of poly(C) linked linearly to poly(U) provided template activity only for its poly(C) moiety. Protein lambda 3 forms complexes with protein lambda 1, as well as with protein lambda 2, and with both lambda 1 and lambda 2, which are sufficiently stable to be precipitated by monospecific antisera. None of these complexes are capable of transcribing either ds- or ssRNA.
Record Date Created: 19930323
Record Date Completed: 19930323

28/7/3 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0299759 DBR Accession No.: 2003-01543 PATENT
Facilitating production of a protein for analyzing, designing and/or modifying an agent that can interact with a viral F protein, comprises expressing a nucleic acid optimized for expression of the protein, using a eukaryotic cell - vector-mediated gene transfer and expression in host cell for recombinant vaccine and gene therapy
AUTHOR: MASON A J; TUCKER S P; YOUNG P R
PATENT ASSIGNEE: BIOTA SCI MANAGEMENT PTY LTD 2002
PATENT NUMBER: WO 200242326 PATENT DATE: 20020530 WPI ACCESSION NO.:
2002-599372 (200264)
PRIORITY APPLIC. NO.: US 252767 APPLIC. DATE: 20001122
NATIONAL APPLIC. NO.: WO 2001AU1517 APPLIC. DATE: 20011122
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Facilitating (M1) production of a protein or its derivative (I) from a negative sense single stranded RNA virus, by expressing a nucleic acid molecule (NAM) encoding (I) in a host cell, where the nucleotide sequence of NAM is optimized for expression by a eukaryotic cell. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an optimized NAM or its derivative, equivalent, analog or mimetic (II); (2) a protein molecule (I) encoded (II); (3) regulating (M2) the functional activity of a viral F protein, where the protein in its non-fully functional form comprises an F2 portion linked, bound or otherwise associated with an F1 portion, and where the F2 portion comprises an intervening peptide sequence, by modulating cleavage of the intervening peptide sequence where excision of a portion of the intervening sequence from the non-fully functional form of the protein up-regulates F protein functional activity; (4) detecting (M3) an agent capable of regulating the functional activity of a viral F protein or its derivative by contacting an eukaryotic cell expressing an optimized NAM with a putative modulatory agent and detecting an altered expression phenotype and/or functional activity; (5) an agent (III) capable of interacting with a viral F protein and modulating a functional activity associated with the viral protein; (6) a viral F protein variant (IV) comprising a mutation in the intervening peptide sequence, where the variant exhibits modulated functional activity relative to wild-type F protein or its derivative, homolog, analog, chemical equivalent or mimetic; (7) a recombinant viral construct (RVC) comprising NAM, where the recombinant viral construct is a effective in inducing, enhancing or otherwise stimulating an immune response to the F protein; and (8) a vaccine comprising RVC. BIOTECHNOLOGY - Preferred Method: In M1, the virus is from family Paramyxoviridae, and sub-family Pneumovirinae, and more preferably the virus is respiratory syncytial virus (RSV). The protein directly or indirectly facilitates fusion of any one or more viral components with any one or more host cells components, where (I) is a F protein or its derivative, which is the Fsol fragment, or is an N, P or SH protein or its derivative. The eukaryotic host cell is preferably a mammalian cell which is a 293 cell, or a Chinese Hamster Ovary Cell, where the optimization is: (a) a codon optimization which comprises modification of an A and/or T comprising codon to express G and C, respectively and the splice site deletion comprises deletion of an RNA splice site; and/or (b) a nucleotide splice site deletion, where the optimized protein encoding nucleic acid molecule further comprises one or more endonuclease restriction sites, where the optimized F protein encoding nucleic acid sequence corresponds to the sequence defined by a sequence of 1725 ((400)3), 1725 ((400)5), 1575 ((400)4), 1575 ((400)6), 726 ((400)556), 1176 ((400)559), 195 ((400)562), given in the specification, or its derivative. Preferred Method: M2 preferably comprises expressing NAM in a host cell, where the cleavage events occur at the cleavage sites defined by the peptide sequence ((400)564) and ((400)563), and where F protein, in its non-fully functional form, comprises the structure X1X2X3. X1 = non-intervening peptide sequence region of the F2 portion; X2 = the intervening peptide sequence region of the F2 portion; and X3 = F1 portion. The regulation is down regulation. In M3, the viral F protein is a non-fully functional form of the protein and the agent modulates cleavage of the intervening peptide sequence, preferably it modulates the site 2 cleavage event. Preferred Agent: (III) is preferably an antagonist which interacts with a sequence selected from 546 sequences, given in the specification, such as (A) - (E) etc. Preferred Variant: (IV) exhibits down-regulated functional activity relative to wild-type F protein, and comprises a mutation in the cleavage site defined by

((400)564), where the mutation comprises amino acid substitution(s) selected from Arg106Gly, Ala107Gln, Arg108Gly, more preferably comprises a sequence of 575 amino acids, given in the specification, or comprises a multiple amino acid deletion from the intervening peptide sequence, where deletion is a partial deletion of the intervening peptide sequence which is (400)569, and where the variant sequence comprises a sequence of 550 ((400)567) amino acids, given in the specification. Preferred Polynucleotide: (II) or its derivative or analog comprises a nucleotide sequence of 3299 or 3450 base pairs, given in the specification. Arg-Ala-Arg-Arg ((400)564) Lys-Lys-Arg-Lys-Arg-Arg ((400)563) Cys-Phe-Ala-Ser-Gly-Gln-Asn-Ile-Thr-Glu (A) Ala-Ser-Gly-Gln-Asn-Ile-Thr-Glu-Glu-Phe (B) Ser-Ala-Val-Ser-Lys -Gly-Tyr-Leu-Ser-Ala (C) Asn-Ala-Val-Thr-Glu-Leu-Gln-Leu-Leu-Met (D) Lys-Lys-Asn-Lys-Cys-Asn-Gly-Thr-Asp-Ala (E) Arg-Ala-Arg-Arg-Glu-Leu-Pro -Arg-Phe-Met-Asn-Tyr-Thr-Leu-Asn-Asn-Ala-Lys-Lys-Thr-Asn-Val-Thr-Leu-Se r (400)569 ACTIVITY - Virucide. MECHANISM OF ACTION - Vaccine (claimed); Gene therapy. No biological data is given. USE - (I), especially F protein, is useful for analyzing, designing and/or modifying an agent capable of interacting with a viral F protein or its derivative and modulating a functional activity associated with the protein, by contacting (I) with a putative agent and assessing the degree of interactive complementarity of the agent with the protein (I). An optimized NAM or its derivative, equivalent, analog or mimetic (II), an agent (III) capable of interacting with a viral F protein and modulating a functional activity associated with the viral protein, or an agent identified using (I) is useful in the manufacture of a medicament utilized in the therapeutic and/or prophylactic treatment of conditions characterized by infection with a negative sense single stranded RNA virus, and for modulating a functional activity associated with a viral F protein in a subject, preferably a mammal, especially a human, where the functional activity is F protein mediated host cell virion fusion and/or virion budding and the modulating is down regulation (all claimed). ADMINISTRATION - Administered respiratorally, intatracheally, nasopharyngeally, intravenously, intraperitoneally, subcutaneously, intracranially, intradermally, intramuscularly, intraoccularly, intrathecally, intracereberally, intranasally, infusion, orally, rectally, or via a drip, patch or implant. Dosage is 0.01 micrograms - 1000 mg/kg. EXAMPLE - Initial attempts to express the respiratory syncytial virus (RSV) F gene sequence in a soluble form (truncated at the transmembrane domain) proved unsuccessful in achieving high levels of expression. The sequence used in the expression vectors was called F(sol) (this differed from the viral sequence in 24/1575 nucleotides where restriction sites had been inserted to allow for easy mutagenesis). The F viral sequence (F(sol)) contained suboptimal codon usage for expression in mammalian cells. A possible eight 3' splice sites were identified, including preceding lariat sequences at four positions. Poly (A) adenylation sites (aataaa (400)570) were also identified at 4 positions. The F natural sequence like the viral sequence was approximately 65 % at rich. Most mammalian expressed genes are less than 50 % AT rich. The DNA sequence encoding the transmembrane form of RSV F. In an attempt to overcome poor expression levels in mammalian cells, a new F sequence was designated that: (i) retained the same encoded amino acid sequence; (ii) used wherever possible optimum codon usage; (iii) removed all potential splice sites and poly A sites; (iv) removed as many CG doublets as these may be methylation sites; (v) designed unique restriction sites to allow cassette mutagenesis; (vi) sequence was checked by secondary structure and any large hairpin loops were destabilized by changing the sequence. The synthetic DNA sequence Fopt (also referred to as F(sol)) was assembled and cloned. Single stranded synthetic DNA fragments of average length 60 bases were annealed and ligated together to produce three fragments - a 631 base pair (bp) Pst 1-Mfe I fragment, a 606 bp Mfe I-Xho I fragment, and a 379 bp Xho I-Bam HI fragment. These gel purified fragments were cloned in pLitmus 38 or a derivative of pLITMUS (pLITMUS 273/279). Especially fragment Pst-Mfe I, Xho I-Bam HI and Mfe-Xho I were sequentially cloned into the cytomegalovirus (CMV) expression vector pCICO or its derivatives (where pCICO is a derivative of pJW4304 which contains a full length CMV promoter and the CMV authentic intron sequence preceding the Pst I site). The 3' terminator used was derived from SV40 early region and this vector also contained the SV40 origin of replication. The plasmid was from the pUC series and contained an ampicillin resistance gene. (pJW4304 was obtained from J. Mullins Dept. of Microbiology, University of Washington, Chapman et al., NAR, 19:3979-3980, 1991). This produced the final clone pCICO.Fopt. pCICO.Fopt was further modified by cloning in a 270 bp EcoRI-Xba I fragment which encodes the transmembrane and cytoplasmic domains of the RSV F protein. (367 pages)

28/7/4 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0284727 DBR Accession No.: 2002-06574 PATENT
New modified RNA virus of genus Morbillivirus, useful as vaccine for protecting humans against Morbillivirus infection, comprises mutations and/or deletions which reduce repression normally caused by V protein - useful for recombinant vaccine for immunization against RNA virus infection
AUTHOR: PARKS C L
PATENT ASSIGNEE: AMERICAN CYANAMID CO 2002
PATENT NUMBER: WO 200200694 PATENT DATE: 20020103 WPI ACCESSION NO.:
2002-139896 (200218)
PRIORITY APPLIC. NO.: US 213655 APPLIC. DATE: 20000623
NATIONAL APPLIC. NO.: WO 2001US19806 APPLIC. DATE: 20010621

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated, recombinantly-generated, nonsegmented, negative-sense, single-stranded RNA virus (I) of the genus Morbillivirus having a mutation in the region corresponding to amino acids 112-134 of Morbillivirus V protein, especially amino acids 113 and 114, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an immunogenic composition (II) comprising (I); (2) reducing the repression caused by a V protein of the genus Morbillivirus, by inserting a mutation in the region corresponding to amino acids 112-134 (especially 113 and 114) of Morbillivirus V protein; (3) an isolated nucleotide sequence encoding a Morbillivirus V protein which has been modified by inserting a mutation in the region corresponding to amino acids 112-134 of a Morbillivirus V protein; (4) a composition comprising a transcription vector comprising an isolated nucleic acid molecule encoding a genome or antigenome of a Morbillivirus, where the portion of the nucleic acid molecule encoding the V protein has been modified to insert a mutation in the region corresponding to amino acids 112-134 of Morbillivirus V protein, together with an expression vector which comprises an isolated nucleic acid molecule encoding the trans-acting proteins N, P and L necessary for encapsidation, transcription and replication, where upon expression an infectious Morbillivirus is produced; and (5) producing an infectious Morbillivirus, by transforming, infecting or transfecting host cells with at least two vectors as above, and culturing the host cells under conditions which permit the co-expression of these vectors so as to produce the infectious Morbillivirus. BIOTECHNOLOGY - Preferred Virus: (I) is measles, canine distemper, rinderpest, pestedes-petits ruminants, dolphin Morbillivirus or phocine distemper virus. (I) has mutation at position 113 and/or 114. (I) further comprises a mutation in or deletion of a portion of a C-terminal region corresponding to amino acids 231-299 of the measles virus V protein, canine distemper virus V protein, rinderpest virus V protein, or corresponding to amino acids 231-303 of the rinderpest virus V protein. The mutation in the C-terminal region is at 233 and 234, from arginine to alanine or aspartic acid. The deletion is chosen from the deletion of amino acids 232-299, 279-299, 267-299, 250-299, 243-299 and 236-299. The deletion extends upstream from the C-terminal region and is from amino acid 229-299. (I) further comprises an attenuating mutation in the 3' genomic promoter region chosen from nucleotides 26 (A to T), 42 (A to T or A to C) and nucleotide 96 (G to A), where these nucleotides are presented in positive strand, antigenomic, message sense, and an attenuating mutation in the RNA polymerase gene chosen from nucleotide changes which produce changes in amino acid residues 331 (I to T), 1409 (A to T), 1624 (T to A), 1649 (R to M), 1717 (D to A), 1936 (H to Y), 2074 (Q to R) and 2114 (R to K). (I) also comprises an attenuating mutation chosen from: (a) for the N gene, nucleotide changes which produce changes in an amino acid residues 129 (Q to K), 148 (E to G) and 479 (S to T); (b) for the P gene, nucleotide changes in amino acid residues 225 (E to G), 275 (C to Y), 439 (L to P); (c) for the C gene, nucleotide changes which produce changes in amino acid residues 73 (A to V), 104 (M to T) and 134 (S to Y); and (d) for the F gene-end signal, the change at nucleotides 7243 (T to C), where these nucleotides are presented in positive strand, antigenomic and message sense. Preferred Composition: (II) further comprises an adjuvant. ACTIVITY - Virucide. MECHANISM OF ACTION - Vaccine. No supporting data is given. USE - (II) is useful for immunizing an individual to induce protection against nonsegmented, negative-sense, single-stranded RNA virus of the genus Morbillivirus (claimed). ADMINISTRATION - Administered by parenteral, oral or topical, preferably intranasal route. Dosage not specified. EXAMPLE - The measles virus N, P and L protein expression clones were each prepared from infected-cell total RNA by reverse transcription and polymerase chain reaction (PCR) amplification (reverse transcription (RT)-PCR) with gene-specific primers, followed by cloning into an appropriate T7 RNA polymerase-dependent expression vector. Vero cells were infected with the Edmonston wild-type strain of measles virus, and when about 70% or more of the cells exhibited a cytopathic effect, RNA was prepared. RT-PCR was performed with avian myoblastosis virus RT and Pwo polymerase. Amplified DNA fragments were cloned into a T7 expression plasmid. Cloned DNAs were checked by cycle-sequencing and nucleotide substitution errors were corrected by oligonucleotide mutagenesis. The initial V protein expression clone was prepared by PCR amplification from an Edmonston wild-type full-length cDNA clone using primers flanking the V protein coding region. The amplified DNA was cloned into the T7 expression vector and the additional G nucleotide residue required to generate the V gene frame shift was added at the editing site by oligonucleotide-directed mutagenesis. Wild-type and mutant V protein expression vectors were also prepared with an influenza virus hemagglutinin (HA) epitope tag. The T7 vector plasmid was modified to include a sequence that included an initiation codon and encoded the HA epitope tag followed by a polylinker. The V protein coding region was cloned with the HA tag at the amino terminus. This served to replace the V protein initiator methionine codon, resulting in the generation of a plasmid, designated pMV-haV-wt. V protein mutants were prepared in the pMV-haV-wt backbone by oligonucleotide-directed or deletion mutagenesis. This modified V protein vector (pMV-haV-wt) also retained the base substitutions that prevented expression of C protein. Testing pMV-haV-wt in minireplicon experiments revealed that the presence of the N-terminal HA tag had no detectable effect on the ability of V protein to repress minireplicon activity (data not shown). Mutations introduced into pMV-haV-wt were directed at specific sequence motifs. One of the mutation resulted in a truncated V protein that lacked the

unique V protein C-terminus containing the cysteine residues (amino acids 231-299 were deleted) (pMV-haV-1). The second mutant haV-5 contained substitution mutations at amino acids 113 and 114. Analysis of the mutant V protein vectors in a minireplication assay indicated that two vectors (pMV-haV-1 and pMV-haV-5) had diminished ability to repress minireplicon activity. (93 pages)

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 File 155:MEDLINE(R)  1966-2003/May W4
     (c) format only 2003 The Dialog Corp.
*File 155: Medline has been reloaded and accession numbers have
changed.  Please see HELP NEWS 155.
 File   5:Biosis Previews(R)  1969-2003/Jun W1
     (c) 2003 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.
 File 357:Derwent Biotech Res.  _1982-2003/Jun W2
     (c) 2003 Thomson Derwent & ISI
*File 357: File is now current.  See HELP NEWS 357.
Alert feature enhanced for multiple files, etc. See HELP ALERT.

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Set   Items   Description
S1     33571   POLYA OR POLY (W)(A  OR ADENYL?)
S2     49128   RNA(W)(VIRUS  OR VIRUSES OR VIRAL)
S3      1504   S1 AND S2
S4        44   VECTOR AND S3
S5        43   RD (unique items)
S6      4239   S1 AND VECTOR?
S7      1617   VIRUS AND S6
S8        19   POLIO? AND S7
S9       379   RNA(W) PHAGE
S10       24   S9 AND VECTOR?
S11     3349   MS2 OR Q(W)BETA
S12    80438   GC OR G(1W)C
S13       42   S11 AND S12
? t s5/7/4 6 18 32 35 38 41
 5/7/4    (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
08755733   20036625   PMID: 10567642
  Characterization  of  infectious Murray Valley encephalitis virus derived
from a stably cloned genome-length cDNA.
  Hurrelbrink R J; Nestorowicz A; McMinn P C
  Department of Microbiology, University of Western Australia, Nedlands, WA
6907, Australia.
  Journal of general virology (ENGLAND)  Dec 1999,  80 ( Pt 12)  p3115-25,
ISSN 0022-1317   Journal Code: 0077340
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  An  infectious  cDNA  clone of Murray Valley encephalitis virus prototype
strain  1-51  (MVE-1-51)  was constructed by stably inserting genome-length
cDNA  into  the low-copy-number plasmid vector pMC18. Designated pMVE-1-51,
the  clone consisted of genome-length cDNA of MVE-1-51 under the control of
a  T7  RNA polymerase promoter. The clone was constructed by using existing
components  of  a  cDNA  library,  in  addition  to cDNA of the 3' terminus
derived  by  RT-PCR of poly(A)-tailed viral RNA. Upon comparison with other
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flavivirus sequences, the previously undetermined sequence of the 3' UTR was found to contain elements conserved throughout the genus FLAVIVIRUS: RNA transcribed from pMVE-1-51 and subsequently transfected into BHK-21 cells generated infectious virus. The plaque morphology, replication kinetics and antigenic profile of clone-derived virus (CDV-1-51) was similar to the parental virus in vitro. Furthermore, the virulence properties of CDV-1-51 and MVE-1-51 (LD(50) values and mortality profiles) were found to be identical in vivo in the mouse model. Through site-directed mutagenesis, the infectious clone should serve as a valuable tool for investigating the molecular determinants of virulence in MVE virus.

Record Date Created: 20000113

Record Date Completed: 20000113

5/7/6 (Item 6 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

08239460 94305417 PMID: 8032269

Replication and translation of cowpea mosaic virus RNAs are tightly linked.

Wellink J; van Bokhoven H; Le Gall O; Verver J; van Kammen A

Department of Molecular Biology, Agricultural University, Wageningen, The Netherlands.

Archives of virology. Supplementum (AUSTRIA) 1994, 9 p381-92, ISSN 0939-1983 Journal Code: 9214275

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The genome of cowpea mosaic virus (CPMV) is divided among two positive strand RNA molecules. B-RNA is able to replicate independently from M-RNA in cowpea protoplasts. Replication of mutant B-transcripts could not be supported by co-inoculated wild-type B-RNA, indicating that B-RNA cannot be efficiently replicated in trans. Hence replication of a B-RNA molecule is tightly linked to its translation and/or at least one of the replicative proteins functions in cis only. Remarkably also for efficient replication of M-RNA one of its translation products was found to be required in cis. This 58K protein possibly helps in directing the B-RNA-encoded replication complex to the M-RNA. In order to identify the viral polymerase the CPMV B-RNA-specific proteins have been produced individually in cowpea protoplasts using CaMV 35S promoter based expression vectors. Only protoplasts transfected with a vector containing the 200K coding sequence were able to support replication of co-transfected M-RNA. Despite this, CPMV-specific RNA polymerase activity could not be detected in extracts of these protoplasts using a poly(A)/oligo(U) assay. These results indicate that, in contrast to the poliovirus polymerase, the CPMV polymerase is not able to accept oligo(U) as a primer and in addition support the concept that translation and replication are linked.

Record Date Created: 19940815

Record Date Completed: 19940815

5/7/18 (Item 18 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

05763906 88117400 PMID: 2828514

Biochemical and biophysical characteristics of Rio Bravo virus (Flaviviridae).

Hendricks D A; Patick A K; Petti L M; Hall A J

Division of Infectious Diseases, Children's Hospital, Boston, Massachusetts 02115.

Journal of general virology (ENGLAND) Feb 1988, 69 (Pt 2) p337-47, ISSN 0022-1317 Journal Code: 0077340

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Rio Bravo (RB) virus has been assigned to the family Flaviviridae on the basis of its antigenic relatedness to other members of this family. RB virus, unlike most members of the Flaviviridae, is believed not to have an arthropod vector. We examined biochemical and biophysical characteristics of RB virus to determine whether it should be assigned to the Flaviviridae and to compare it with arthropod-borne flaviviruses. Purified RB virus banded at a density of 1.18 g/ml in sucrose and had a sedimentation coefficient of about 200 S. Virions, negatively stained with ammonium molybdate, were spherical, had diameters of 42 nm, and appeared to be surrounded by envelopes bearing surface projections. The loss of infectivity after infectious virus was incubated with diethyl ether or sodium deoxycholate confirmed the presence of envelopes. Partially purified RB virions contained single-stranded RNA, lacking 3' poly(A) tracts, that sedimented in a 15% to 30% sucrose gradient as one discrete band with a sedimentation coefficient of about 40 S. Most of the viral proteins in preparations of purified virus and in immunoprecipitates had similar electrophoretic mobilities and glycosylation patterns to known flavivirus proteins. Therefore, they were assigned the following tentative designations using the nomenclature for flavivirus proteins: gp52 and gp47, envelope proteins; gp46, non-structural protein 1; p25, gp20(prM), precursor to membrane protein; gp less than 18K. Putative core and membrane proteins were not identified. These physical and biochemical characteristics of RB virus are remarkably similar to those of the arthropod-borne members of the Flaviviridae and they confirm the classification of RB virus in this family. This is the first report of biochemical and physical properties of a non-arthropod-borne member of the Flaviviridae.

Record Date Created: 19880321

Record Date Completed: 19880321

5/7/32 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12736947 BIOSIS NO.: 200000490570
Genetic engineering of influenza and other negative-strand RNA viruses containing segmented genomes.
BOOK TITLE: Advances in Virus Research
AUTHOR: Neumann Gabriele(a); Kawaoka Yoshihiro(a)
BOOK AUTHOR/EDITOR: Maramorosch Karl; Murphy Frederick A; Shatkin Aaron J: Authors
AUTHOR ADDRESS: (a)Department of Pathobiological Sciences School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin, 53706**USA
JOURNAL: Advances in Virus Research 53p265-300 1999
MEDIUM: print
BOOK PUBLISHER: Academic Press Inc., 525 B Street, Suite 1900, San Diego, CA, 92101-4495, USA
Academic Press Ltd., 24-28 Oval Road, London, NW1 7DX, UK
ISSN: 0065-3527 ISBN: 0-12-039853-2 (cloth)
DOCUMENT TYPE: Book
RECORD TYPE: Citation
LANGUAGE: English
SUMMARY LANGUAGE: English

5/7/35 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0299758 DBR Accession No.: 2003-01542 PATENT
Vaccine for preventing/treating diseases caused by pathogen which infects/avoids destruction by macrophages, has vector having nucleotide sequence encoding pathogen-derived antigen which generates immune response - vector-mediated gene transfer and expression in host cell for nucleic acid vaccine and gene therapy
AUTHOR: GAULDIE J; BRACIAK T
PATENT ASSIGNEE: GAULDIE J; BRACIAK T 2002
PATENT NUMBER: US 20020086837 PATENT DATE: 20020704 WPI ACCESSION NO.:
2002-635685 (200268)
PRIORITY APPLIC. NO.: US 742892 APPLIC. DATE: 20001221
NATIONAL APPLIC. NO.: US 742892 APPLIC. DATE: 20001221
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A vaccine (I) useful in preventing and treating diseases caused by a pathogen capable of infecting, or avoiding destruction by, macrophages, comprising a vector which has a nucleotide sequence encoding at least one antigen derived from the pathogen, where the antigen is capable of generating an immune response in its recipient, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a kit comprising a container and one or more patches, having disposed in it a vector comprising a nucleotide sequence encoding an antigen derived from a pathogen capable of infecting, or avoiding destruction by macrophages; (2) an article of manufacture comprising solution of (I) disposed within a tube, vial, bottle, can or syringe; and (3) cosmetically improving the appearance of a person's skin who is suffering from acnes vulgaris, by obtaining a composition comprising a mixture of a vector that comprises at least one nucleotide sequence encoding an antigen derived from Propionibacterium acnes, and a cosmetic agent, and administering the composition to the person. BIOTECHNOLOGY - Preferred Vaccine: The vector comprises naked DNA, a recombinant viral vector (such as adenovirus, adeno-associated virus, herpes virus, vaccinia or RNA viruses), or a combination of both, and a nucleotide sequence encoding an adjuvant, especially a cytokine such as interleukin-2 (IL-2), IL-12 or both. The antigen is a lipase gene, a hyaluronidase gene, a phosphatase gene, their fragments, or their combinations. (I) is in the form of an aqueous solution, and further comprises a nucleotide sequence encoding a co-stimulatory molecule which comprises a B7 protein, a CD40 protein or both. ACTIVITY - Antibacterial; Antiseborrheic; Dermatological; Antiinflammatory; Fungicide; Protozoacide. No biological data is given. MECHANISM OF ACTION - Vaccine; Inducer of immune response. The effect of vaccine containing AdE1 lipase (Ad5E1PBAL) vector on Balb/c female mice (8-14 week) was evaluated. Mice were immunized intramuscularly with 2x10 to the power 9 plaque forming unit (pfu) in 50 micro-l saline of AdE1 lipase (Ad5E1PBAL) or control (empty) vector (DL70-3) intramuscularly (I.M.) on left hind leg. 7 days later disease was induced by injection of 100 micro-l of 1x10 to the power 9 colony forming unit (cfu)/ml of P. acnes I.M. in phosphate buffered saline (PBS) on left rear flank. All recombinant viruses were propagated and purified for the Ad5E1PBAL vector. Control vector DL70-3 was an Ad5 variant deleted in the E1 region. All reactions were measured by caliper sizing. The results demonstrated that pre-immunization with lipase of P. acnes provided protections from P. acnes challenge. USE - (I) is useful for treating or preventing a disease caused by a pathogen capable of infecting, or avoiding destruction by macrophages, where the pathogen includes Propionibacterium acnes, Listeria monocytogenes, Salmonella typhimurium, Neisseria gonorrhoea, Mycobacterium avium, M. tuberculosis, M. leprae, Brucella abortus, Candida albicans, Leishmania major (claimed). (I) is useful for eliciting protective immune responses against colonization of the bacterium in skin follicles. ADMINISTRATION - (I) is administered by oral, intravenous, intramuscular, subcutaneous, aerosol, ocular, rectal, intraperitoneal,

intrathecal, or preferably transcutaneous route by applying the vector to a patch, and adhering the patch to skin of the recipient (claimed). No dosage is given. ADVANTAGE - Adenovirus vector in (I) is highly efficient in transferring genetic material to the target cell, has ability to carry large segments of DNA and has the ability to infect non-dividing cells. The gene expression of the vector is transient in the target cell due to lack of integration of the viral DNA into the host cell DNA, which is highly advantageous compared to other vectors which integrate into the chromosomes and cause insertional inactivation or mutation of genes. EXAMPLE - Construction of recombinant plasmids and adenovirus containing a functional coding gene for Propionibacterium acnes lipase was as follows. To rescue P. acnes lipase sequences into translatable minigene cassette, an oligonucleotide was designed containing 5' flanking restriction enzyme sites for BamHI and HindIII, followed subsequently by a sequence coding for the consensus optimal ribosomal translation initiation site, and bases incorporating first 30 nucleotides of the coding sequence for P. acnes lipase gene. The sequence of 5' oligonucleotide was: GCGGATCCAAGCTTGCCGCCG-CCATGAAGATCAACGCAC-GATTCGCCGTC. An additional oligonucleotide containing bases complementary to 3' end of P. acnes lipase gene flanked by residues containing stop codons to provide a translational termination signal and restriction site XhoI was created. The sequence of 3' oligonucleotide was CGCCCGCTCGAGCTA-TCATGCAGCATCCGTG GTGGATACGGGCAG. Additional nucleotides were incorporated in the design of the 5' and 3' oligonucleotides to accommodate for restriction enzyme cleavage activity at blunt ends of DNA. Polymerase chain reaction (PCR) reactions were carried out using the 5' and 3' designed oligonucleotides with genomic DNA isolated from P. acnes bacteria. PCR fragment was isolated and subcloned by the blunt end ligation into pCR-Blunt. The lipase sequence was rescued from the blunt vector by KpnI and XhoI digest and cloned into the sites in the pDK6 vector. This construction placed the transgene under the control of the murine cytomegalovirus (mCMV) promoter and provided polyadenylation signals from the simian virus 40 (SV40). To obtain the resultant adenovirus vector expressing P. acnes lipase gene, pDK6PBAL DNA was cotransfected with pBHG10 into 293 cells using standard adenovirus rescue protocols. One viral plaque was identified by restriction enzyme digest, Southern blot and by sequence to contain P. acnes lipase gene sequence and was designated as Ad5E1PBAL vector. This recombinant vector was propagated in 293 cells and purified. (12 pages)

5/7/38 (Item 6 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0284076 DBR Accession No.: 2002-05923 PATENT

Minimum plasmid-based system for generation of infectious RNA viruses, preferably influenza viruses, comprises plasmids containing RNA polymerase I and II promoter - plasmid-mediated influenza virus hemagglutinin or neuraminidase and RNA-polymerase-I or RNA-polymerase-II promoter gene transfer and expression in 293T cell-Madin-Darby dog kidney cell for recombinant vaccine and virusinfection therapy
AUTHOR: HOFFMANN E
PATENT ASSIGNEE: ST JUDE CHILDREN'S RES HOSPITAL 2001
PATENT NUMBER: WO 200183794 PATENT DATE: 20011108 WPI ACCESSION NO.: 2002-075166 (200210)
PRIORITY APPLIC. NO.: US 200679 APPLIC. DATE: 20000428
NATIONAL APPLIC. NO.: WO 2001US13656 APPLIC. DATE: 20010427
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Minimum plasmid-based system (I) comprises a set of plasmids, each comprising one autonomous viral genomic segment (VGS), where the vcDNA corresponding to VGS is inserted between RNA polymerase I (pol I) promoter and terminator sequences. vRNA is expressed and inserted between a RNA polymerase II (pol II) promoter and a polyadenylation signal, resulting in expression of viral mRNA. DETAILED DESCRIPTION - Minimum plasmid-based system (I) for generation of infectious negative strand RNA viruses from cloned viral cDNA (vcDNA) comprises a set of plasmids, each comprising one autonomous viral genomic segment (VGS), where the vcDNA corresponding to VGS is inserted between RNA polymerase I (pol I) promoter and terminator sequences. vRNA is expressed and inserted between a RNA polymerase II (pol II) promoter and a polyadenylation signal, resulting in expression of viral mRNA. INDEPENDENT CLAIMS are also included for the following: (1) an expression plasmid (II) comprising an RNA polymerase I (pol I) promoter and pol I terminator sequences, which are inserted between an RNA polymerase II (pol II) promoter and a polyadenylation signal; (2) a host cell (III) comprising (I); (3) vaccinating a subject against a negative strand RNA virus infection, by administering a vaccine prepared by purifying a virion produced by culturing (III) that permits production of viral proteins and vRNA or cRNA; (4) a composition comprising a negative strand RNA virus virion, where viral internal proteins of the virion are from a virus strain well adapted to grow in culture or from an attenuated strain, or both and viral antigen proteins of the virion are from a pathogenic virus strain; and (5) a composition comprising a negative strand RNA virus virion produced by culturing (III). BIOTECHNOLOGY - Preparation: (I) is prepared according to standard methodologies. Preferred System: The pol I promoter is proximal to the polyadenylation signal and the pol I terminator sequence is proximal to the pol II promoter or vice versa. The plasmid further comprises a negative strand RNA virus viral gene

segment inserted between the pol I promoter and the termination signal. The negative strand RNA virus is a member of Orthomyxoviridae virus family and the virus is an influenza A or B virus. The viral gene segment encodes a protein chosen from a viral polymerase complex protein, M protein and NS protein, where the genes are derived from a strain well adapted to grow in cell culture or from an attenuated strain or both. The viral genomic segments comprise gene which encodes a protein such as hemagglutinin (HA), neuraminidase (NA) or both. The genes are from a pathogenic influenza virus. The system comprises one or more plasmids having a map of pHW241-PB2, pHW242-PB1, pHW243-PA, pHW244-HA, pHW245-NP, pHW246-NA, pHW247-M, and pHW248-NS and pHW181-PB2, pHW182-PB1, pHW183-PA, pHW184-HA, pHW185-NP, pHW186-NA, pHW187-M and pHW188-NS. (II) corresponds to a plasmid having a map of pHW2000, pHW11 and pHW12. ACTIVITY - Virucide; immunostimulant. No supporting data is given. MECHANISM OF ACTION - Vaccine. No supporting data is given. USE - (III) comprising (I), is useful for producing a negative-strand RNA virus virion, Orthomyxoviridae virion, influenza virion or a pathogenic influenza virion, by culturing (III) that permits production of the viral proteins and vRNA or cRNA. (I), (III) and methods of producing virions are particularly suitable for preparing an attenuated RNA virus-specific vaccine, by purifying and inactivating the produced virions. The vaccine produced is useful for vaccinating a subject against negative strand RNA virus infection. (I) is also useful for generating an attenuated negative RNA virus, by mutating one or more viral genes in the plasmid-based system and determining whether infectious RNA viruses produced by the system are attenuated (claimed). Furthermore, (I) may be used for producing negative strand segmented viruses, nonsegmented negative strand RNA viruses and positive strand RNA viruses. (I) facilitates the design and recovery of both recombinant and reassortment influenza A viruses and is also applicable to the recovery of other RNA viruses entirely from cloned cDNA. ADMINISTRATION - Administration is intramuscular or intranasal (claimed). Dosage not specified. ADVANTAGE - (I) limits the total number of plasmids required to establish the system in a host cell, eliminating the need for helper virus, selection process and permitting efficient generation of reassortment viruses. By using a single viral cDNA for both protein synthesis and genomic RNA synthesis, (I) allows the development of vaccines quickly and cheaply. EXAMPLE - The pHW2000 cloning vector contained 225 bp of the human pol I promoter and 33 bp of the murine terminator sequence separated by two BsmBI sites. The pol I promoter and terminator elements were flanked by a truncated immediate-early promoter of the human cytomegalovirus and by the polyadenylation signal of the gene encoding bovine growth hormone. The eight plasmids containing the cDNA of the virus A/WSN/33 (H1N1) (pHW181-PB2, pHW182-PB1, pHW183-PA, pHW184-HA, pHW185-NP, pHW186-NA, pHW187-M, and pHW188-NS) were constructed by inserting ApaI-SalI fragments (with viral cDNA and polI promoter and terminator sequences) of the plasmids pPolI-WSN-PB2, pPolI-WSN-PB1, pPolI-WSN-PA, pPolI-WSN-NP, pPolI-WSN-HA, pPolI-WSN-NA (Neumann et al., Proc. Natl. Acad. Sci. USA 1999, 96:9345), pHW127-M and pHW128-NS into the ApaI-salI vector fragment of pHW2000. The eight expression plasmids contained the eight cDNAs of A/WSN/33 (H1N1). Eight plasmids (1 microg of each plasmid) were cotransfected into transiently cocultured 293T-Madin-Darby canine kidney (MDCK) cells. Both cell lines were cocultured in one-cell culture well before transfection and for replication efficiency of influenza A viruses. After 48 and 72 hours, the MDCK cells showed a virus-induced cytopathic effect, but no cytopathic effect was observed after transfection of seven plasmids without the PB1-expression construct. 24 hours post-transfection cell supernatant contained 1x103 viruses/ml and 2x107 infectious viruses were generated 72 hours post-transfection/ml. To verify that the generated virus was the designed A/WSN-virus, the cDNA was produced for the NS gene by reverse transcriptase-polymerase chain reaction (RT-PCR). The generation of two fragments after digestion with the restriction endonuclease NcoI and sequence analysis of the amplified fragment confirmed that the recovered virus was the designed A/WSN-virus. These findings showed that the pol I and pol II-driven synthesis of vRNA and mRNA from eight templates resulted in the generation of infectious influenza virus. (99 pages)

5/7/41 (Item 9 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0238137 DBR Accession No.: 99-08238
RNA virus vectors: where are we and where do we need to go? - a review
AUTHOR: Palese P
CORPORATE AFFILIATE: Mount-Sinai-Sch.Med.
CORPORATE SOURCE: Department of Microbiology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, USA.
JOURNAL: Proc.Natl.Acad.Sci.U.S.A. (95, 22, 12750-52) 1998
ISSN: 0027-8424 CODEN: PNASA6
LANGUAGE: English
ABSTRACT: RNA viruses and their components are now being used to express foreign genes. Sindbis virus is an alpha virus containing a 12 kb ss positive-sense, capped and polyadenylated RNA genome. When the genomic naked RNA of the virus is introduced into cells, infectious virus forms. 2 methods have been used to express foreign proteins from Sindbis virus constructs: a self-replicating, self-limiting replicon made by replacing the genes for the virion structural proteins was replaced with that of a reporter gene; and chimeric virus is constructed by introducing a subgenomic promoter, which drives the expression of the heterologous protein, where these replicons and

vectors amplify to high levels and will kill the transfected/infected cells. Methods to circumvent the cytopathic properties of these vector systems are described. Second generation bipartite vectors (DI/replicon systems), which are noncytopathic and express levels as high as 30 ug foreign protein per million cells have been formed. RNA viruses may be used for gene therapy since they meet strict safety guidelines, but their success will depend on their ability to target the vectors to specific cells. (39 ref)

? t s8/7/12

8/7/12 (Item 4 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0292742 DBR Accession No.: 2002-14589 PATENT

Novel bacteriophage vector chimerized with polypeptides or proteins of eukaryotic viruses, useful for gene transduction of eukaryotic cells, and in gene therapy - phage, phagemid or plasmid-mediated gene transfer and expression in host cell for gene therapy

AUTHOR: SAGGIO I; SALONE B; DI GIOVINE M; YURI M

PATENT ASSIGNEE: CONSORZIO INTERUNIVERSITARIO NAZ FISICA 2002

PATENT NUMBER: WO 200224934 PATENT DATE: 20020328 WPI ACCESSION NO.:
2002-404957 (200243)

PRIORITY APPLIC. NO.: IT 20002073 APPLIC. DATE: 20000922

NATIONAL APPLIC. NO.: WO 2001IB1742 APPLIC. DATE: 20010921

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A bacteriophage vector (I) for gene transduction of eukaryotic cells, where (I) is chimerized with polypeptides or proteins of eukaryotic viruses, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) preparation (M1) of chimeric phage particles involves introducing of a DNA sequence encoding a viral protein or polypeptide of an eukaryotic virus into the genome of a bacteriophage or into a phagemid or into a plasmid, transforming a bacterial host for the production of chimeric phage particles, optionally infecting with the whole phage, and purifying the chimeric phage particles; (2) a chimeric phage particle (II) obtained by M1; (3) identifying (M2) binding and internalization mutants of a penton-base adenoviral protein, involves random mutagenesis of the nucleotide sequence encoding penton-base adenoviral protein in a phagemid vector or in a phage genome or in a plasmid, production of chimeric phage particles comprising the mutagenized sequences, contacting the chimeric phage particles with cells expressing integrins in selective conditions, and recovery of chimeric phagemid vectors selected after specific adhesion or internalization; (4) a composition (III) comprising a physiologically acceptable liquid and (I) or (II); and (5) use of a nucleotide sequence (IV) encoding penton-base adenoviral protein (SwissProt IDN:P03276), or its fragments, serotype variants or conservative mutations, for the preparation of chimeric vectors for the transduction of eukaryotic cells. BIOTECHNOLOGY - Preferred Vector: (I) is a chimeric vector, and further comprises a therapeutic gene under the control of a transcription promoter. In (I), the viral polypeptides or proteins are structural proteins of eukaryotic viruses such as influenza virus, herpes viruses, retroviruses, polioma virus, SV40, adenoviruses, adeno-associated viruses, vaccinia virus, or lentivirus, or VP22 of herpes virus HSV, hemagglutinin of influenza virus, adenoviral penton-base. The viral polypeptides or protein comprises amino acids 1-571 of adenoviral penton-base (SwissProt IDN:P 03276), at least one polypeptide fragment, or amino acids 295-380 of the adenoviral penton-base or its serotype variants or its conservative mutants. The polypeptide fragment comprise 6-10 consecutive amino acids of adenoviral penton-base protein. (I) is a filamentous phage M13 or its phagemids or lambda bacteriophage or its phagemids or plasmids. Preferred Method: In M1, the genome of the bacteriophage or phagemid or the plasmid further comprises a therapeutic gene under the control of a transcription promoter. The introduction into the genome of the bacteriophage or into the phagemid or into the plasmid occurs at the level of the DNA sequence encoding for a structural protein of the phage particle. The structural phage protein is chosen from pIII or pVIII capsidic protein of M13, D capsid protein of lambda phage or V protein of the tail of lambda phage, preferably M13 capsidic protein pIII or the capsidic D protein of lambda phage. The DNA sequence encoding viral proteins or polypeptides comprises the nucleotide sequence encoding amino acids 1-571, 295-380, or at least 6 consecutive amino acids of adenoviral penton-base or its conservative mutants or its serotype variants. In M1, the genome of the bacteriophage, is the genome of the filamentous phage M13mp or one of its phagemids or of the lambda phage or its phagemids or plasmids. Preferred Sequence: In (IV), the fragment comprises the sequence encoding amino acids 295-380 of penton-base adenoviral protein or its serotype variants. ACTIVITY - None given. No biological data is given. MECHANISM OF ACTION - Gene therapy. No biological data is given. USE - (I) or (II) is useful for transduction of eukaryotic cells, preferably expressing at least one type of integrin such as alphavbeta3, alphavbeta5, alpha5beta1, or alpha3beta1 integrins, by contacting (I) or (II) with eukaryotic cells at a temperature between 25-39 degrees Centigrade, so that the vector is internalized. (IV) is useful for the preparation of (I) (claimed). (I) is useful in gene therapy, and for identifying binding and internalization mutants of a penton-base adenoviral protein. ADVANTAGE - (I) can be amplified and produced in their natural hosts, i.e. bacteria, thus greatly simplifying all the steps which are necessary in order to obtain high-titer virus stock and which are carried out in mammalian cells for eukaryotic viral vectors. (I) is safer because, since their natural hosts are bacteria, they do not have sequences

which might potentially interfere with eukaryotic cell functions, and which might therefore be potentially dangerous, as it happens for viruses whose natural hosts are humans and mammals. EXAMPLE - Production of chimeric phages for penton-base adenoviral protein was as follows: The gene encoding the complete sequence of penton-base adenoviral protein (Pb) (SwissProt IDN P03276) and its central domain (DELTAPb aa 286-393) were amplified by polymerase chain reaction from the DNA of Ad2 with the following pairs of primers: (Pb 1-517): 5'-GATCGTCGACATGCAGCGCGCGGCGATGTATGAGG-3', 5'-TGACGCGGCGCCCTAAAAAGTGCGG CTCGATAGGACGCGC-3', and (Pb 286-393): 5'-GATCGTCGACCTGTTGGATGTGGACGCCTA CCAGGCA-3', 5'-TGACGCGGCCGCCCTATAGGTTGTAACTGCGTTTCTTGCTGTC-3' and introduced into SalI-NotI site of pHenDELTA phagemid in a position corresponding to the C-terminal portion of pIII capsidic protein. Control phages containing no adenoviral sequence were also prepared. The expression box CMV-GFP-polyA for the expression of the eukaryotic protein green fluorescent protein was obtained by digestion of pITRUF5-N plasmid with EcoRI and SalI enzymes, made blunt-ended and then sub-cloned in the recombinant phagemids Pb(1-571)-pHenDELTA and Pb(286-393)-pHenDELTA. The sequence and extension of the deletion of penton-base adenoviral protein in the chimeric phage were designed considering several parameters for the production of DELTAPb chimeric phage: minimization of insert size to limit possible interference in the assembly of DNA and of the phage capsid, inclusion within the fragment of Pb-binding pattern RGD for integrins, and stability of deleted insert, evaluated both on the basis of literature data and by predicting the secondary structure with predict protein. The phages were prepared by transformation of Escherichia coli XI1-blue, super-infected with helper phage M13 KO7 and the secreted phage form was PEG-precipitated from the supernatant of E. coli, then further purified by ultracentrifugation on CsCl gradient. The expression of pb and DELTApb adenoviral protein or the phage capsid was verified by western-blotting of the chimeric phage particles purified from the culture medium of infected bacteria. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotting onto nitrocellulose, the filter was saturated with TBS/5% powder milk/0.05% Tween 20 (TBSMT) and incubated with an anti-Pb rabbit polyclonal antibody diluted 1:1000 in TBSMT, and the bound antibody was detected. The results of the Western blot analysis showed that the chimeric proteins were expressed on the phage with the expected molecular weight and that the chimeric proteins were also recognized by specific anti-penton-base antibodies. The chimeric proteins on the phage capsid were further quantified using as controls a sample consisting of 1.2 x 10 (to the power of 6) (corresponding 7.2 x 10 (to the power of 6) molecules of Pb) and a control consisting of 1.2 x 10 (to the power of 6) phage particles (corresponding to 4.8 x 10 (to the power of 12) molecules of pIII) and measuring the corresponding signal intensity. The relative quantification showed that the chimeric protein DELTAPb-pIII was expressed in 1/20 particles of the phage stock, whereas the chimeric protein corresponding to the complete form of penton-base Pb-pIII protein was expressed in 1/90 of the phage stock.(41 pages)

? t s13/7/10 31 37 42

13/7/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06168105 89183595 PMID: 2928109
Codon usage and secondary structure of MS2 phage RNA.
Bulmer M
Department of Statistics, Oxford, UK.
Nucleic acids research (ENGLAND) Mar 11 1989, 17 (5) p1839-43,
ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
MS2 is an RNA bacteriophage (3569 bases). The secondary structure of the RNA has been determined, and is known to play an important role in regulating translation. Paired regions of the genome have a higher G+C content than unpaired regions. It has been suggested that this reflects selection for high G+C content to encourage pairing, but a re-analysis of the data together with computer simulation suggest that it is an automatic consequence in any RNA sequence of the way it folds up to minimise its free energy. It has also been suggested that the three registers in which pairing can occur in a coding region are used differentially to optimise the use of the redundancy of the genetic code, but re-analysis of the data shows only weak statistical support for this hypothesis.
Record Date Created: 19890509
Record Date Completed: 19890509

13/7/31 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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06672659 BIOSIS NO.: 000087114836
CODON USAGE AND SECONDARY STRUCTURE OF MS2 PHAGE RNA
AUTHOR: BULMER M
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JOURNAL: NUCLEIC ACIDS RES 17 (5). 1989. 1839-1844. 1989
FULL JOURNAL NAME: Nucleic Acids Research
CODEN: NARHA
RECORD TYPE: Abstract

LANGUAGE: ENGLISH
ABSTRACT: MS2 is an RNA bacteriophage (3569 bases). The secondary structure of the RNA has been determined, and is known to play an important role in regulating translation. Paired regions of the genome have a higher G+C content than unpaired regions. It has been suggest that this reflects selection for high G + C content to encourage pairing, but a re-analysis of the data together with computer simulation suggest that it is an automatic consequence in any RNA sequence of the way it folds up to minimise its free energy. It has also been suggested that the three registers in which pairing can occur in a coding region are used differentially to optimise the use of the redundancy of the genetic code, but re-analysis of the data shows only weak statistical support for this hypothesis.

13/7/37 (Item 22 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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SITE DIRECTED MUTAGENESIS IN DNA GENERATION OF POINT MUTATIONS IN CLONED
BETA GLOBIN COMPLEMENTARY DNA AT THE POSITIONS CORRESPONDING TO
AMINO-ACIDS 121 TO 123
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ZUERICH, SWITZ.
JOURNAL: J MOL BIOL 124 (2). 1978 343-358. 1978
FULL JOURNAL NAME: Journal of Molecular Biology
CODEN: JMOBA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH
ABSTRACT: The principle of site-directed mutagenesis, previously applied to the RNA of bacteriophage Q.beta., was utilized to generate nucleotide transitions in a predetermined region of DNA. Plasmid P.beta.G, which contains an almost complete DNA copy of rabbit .beta. globin mRNA, was nicked at the EcoRI site which is located within the globin gene, in a region corresponding to amino acids 121 and 122. Substrate-limited nick translation using DNA polymerase I and N4-hydroxydCTP, dCTP and dATP led to the replacement of TMP residues by the nucleotide analog in the immediate vicinity of the nicks. The substituted DNA was amplified in vivo, treated with EcoRI and retransfected [Escherichia coli]. Of the amplified DNA, 1.9% was EcoRI-resistant. Nucleotide sequence analysis of the critical region of 6 EcoRI-resistant isolates revealed that 2 plasmids had 1, 3 had 2 and 1 had 3 A .cntdot. T .fwdarw. G .cntdot. C transitions, all located within the substituted region. No point mutations (< 3 .times. 10-3%) were found in control preparations, but a small number of deletion mutants, lacking the EcoRI site, were isolated.

13/7/42 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
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0024289 DBR Accession No.: 84-07564
High-level expression of human interferon-gamma in Escherichia coli under control of the pl promoter of bacterio-phage lambda - plasmid expression vector construction
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CORPORATE SOURCE: Laboratory of Molecular Biology, State University of Ghent, Ledeganckstraat 35, B-9000 Ghent, Belgium.
JOURNAL: Gene (28, 1, 55-64) 1984
CODEN: GENED6
LANGUAGE: English
ABSTRACT: The application of the PL promoter of phage lambda to achieve high-level synthesis of mature IFN-gamma in Escherichia coli is described. The recombinant plasmids contain either a ColE1-type replication origin or the origin of a runaway replication derivative of plasmid R1drd19. Ribosome-binding sites derived from the phage MS2 replicase gene or from the E.coli trp attenuator were used. The source of human IFN-gamma coding sequence was plasmid pHIIF-SV-gamma1. The IFN-gamma sequence including G-C tails was excised with BamHI and inserted in pPLc28 giving pPLcHIIF-5. This was cleaved with AvaII and HindIII and the fragment containing IRN-gamma coding sequence was ligated to a synthetic double-stranded oligonucleotide. The blunt ended HindIII IFN-gamma fragment was then obtained and linked to either of 2 expression vectors: pPLc245 or pPLc236trp. IFN levels of up to 25% of total cellular protein can be achieved using these vectors. The highest levels were obtained when a terminator of transcription was cloned downstream from the IFN-gamma sequence. (31 ref)
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